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Discussion Meeting on Growth Factors

24 *June* 1937

Opening Address

BY F. KÖGL, *Utrecht*

on

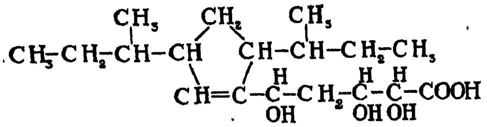
PLANT GROWTH HORMONES

The growth of a plant cell may be separated schematically into the following stages: cell division, plasmatic growth and cell elongation. The definite proof that the growth of plant cells is regulated by plant hormones has first been found for the process of cell elongation. The term *auxins* is used to designate these hormones.

The function of auxins in plants is not limited to cell elongation. Botanical investigations have shown that they also play a role in phototropism and geotropism, that they stimulate root development, induce cambial growth and prevent premature outgrowth of axillary buds. Auxins therefore regulate plant growth in many ways.

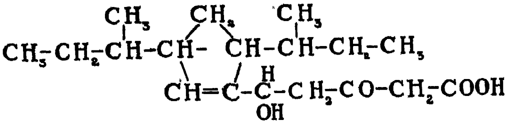
The isolation of auxins has been carried out successfully in the laboratory at Utrecht, by making use of the Went test reaction. They have been isolated in crystalline form from various sources: from urine, maize oil, malt and from yeast. Surprisingly, there are two types of auxins, entirely unrelated chemically. To the first type belong auxins *a* and *b*; these are acids of the empirical formulas $C_{18}H_{32}O_5$ and $C_{18}H_{30}O_4$ respectively (activity 5×10^{10} AE/g.). On the other hand, heteroauxine, the second type, which was found later, contains nitrogen and is identical with the long known and easily synthesized β-indolylacetic acid (activity $2{\cdot}5 \times 10^{10}$ AE/g.).

The formulas of auxin-*a* and *b* (I and II) have been substantiated by a great many degradation reactions, and in addition the constitution of the greater part of the molecule has been definitely established through the synthesis of auxin-glutaric acid III:



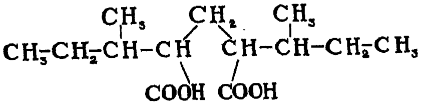
Auxin—a.

I



Auxin—b

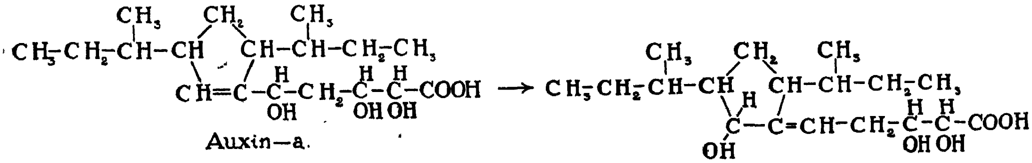
II



Auxin Glutaric acid

III

The auxins lose their activity on standing through a spontaneous allylic rearrangement:



Auxin—a.

Auxin-*a*-lactone also loses its activity on standing. In contrast to the free acid the rearrangement of the lactone can also be brought about by ultraviolet light, in which reaction sensibilizators apparently play a role. The constitution of the reaction product (lumi-auxin-*a*-lacton) is not yet completely clear. According to the current view, positive phototropism is explained by the effect of light in causing an increased auxin concentration in the shaded side. However, it must remain for further investigations to show whether the deficiency of auxin in the lighted side cannot be due in part at least to the formation of lumi-auxin-*a*-lacton.

That grass tips produce auxin-*a* and not *β*-indolylacetic acid has in various ways been proved with a very high degree of probability.

During the last 3 years the easily available synthetic *β*-indolylacetic acid has been of great importance in botanical research. In the same manner as auxin-*a* it causes root formation on stem cuttings.

It is remarkable that this reaction is less dependent on a specific structure than the seedling tip test. Investigators in the Boyce Tompson Institute have found a great number of compounds only distantly related to *β*-indolylacetic acid, which are active in this regard, for example benzol, naphthaline, anthracene, fluorene, etc., substituted acetic acids. In general, the situation seems to be similar to the existence of numerous oestrogenic compounds found by Dodds and co-workers, which have little in common chemically with the hormones of the oestrone group.

Although it was originally believed that auxins influence only cell elongation, it has since been found that they bring about cell division in root formation, cambial growth (Snow) and in callous formation (Laibach and others), but only in much higher concentrations. In the plasmatic growth which follows cell division and which is primarily characterized by increase in protoplasm, the daughter cells grow to the size of the mother cells. It has not yet been possible to determine whether substances which cause cell multiplication in yeast influence cell division, plasmatic growth, or possibly both processes. As oat seedlings for auxins, so yeast is the classic test object for "bios"-growth factors.

After Wildiers's fundamental discovery (1901), only in the last 10 years has the investigation of bios made decisive progress. In 1929 Lash Miller and co-workers showed that bios is of multiple nature, and that one of the factors is identical with meso-inositol. Shortly thereafter R. J. Williams reported that aneurin (vitamin B_1) functions as a bios factor for a certain strain of yeast. In 1928 A. M. Copping first called attention to the differing requirements of wild and highly cultivated strains of yeast.

A further bios factor—biotin—was isolated from egg yolk in the laboratory at Utrecht in 1934. This is by far the most expensive growth factor known. For its isolation a 3 millionfold enrichment is necessary, which involves a rather difficult and tedious scheme of fractionation. By this method not free biotin is obtained but the beautifully crystalline methylester.

It has recently been shown that this crystalline product contains sulphur in addition to oxygen and nitrogen. On the basis of the analytical results, the formula of the ester is $C_{11}H_{18}O_3N_2S$. It is very interesting that besides aneurin still another bios factor is shown to be a sulphur-containing amine. The characterization of the functional groups is still in progress; but it has already been possible to obtain a crystalline degradation product.

Biotin is one of the most active biological compounds known; it can be detected in the yeast test even in a dilution of 1 in 4×10^{11}.

Another bios component is factor Z, which accelerates the fermentation rate of living yeast. Starting from yeast, we have also been able to enrich this factor greatly.

It is certain that the number of bios factors will still be further increased. R. J. Williams and co-workers have established the stimulating effect of β-alanin on yeast growth. These investigators have for some years studied a factor which received the name pantothenic acid, because of its ubiquitous occurrence. In this regard it is similar to biotin. The preparations of this factor from liver have been greatly purified but the factor has not yet been obtained in crystalline form. Lash Miller has stated that besides the abovementioned factors, *l*-leucin and a chemically undefined, labile factor (bios V) are components of the bios complex.

As was to be expected, similar situations are met in the growth requirements of other lower organisms. The effect of aneurin (vitamin B_1) on *Phycomyces Blakesleeanus* and other mucorinae is known through the work of Burgeff and Schopfer. We have recently established that *Polyporus adustus* also requires aneurin for its growth. The situation in regard to *Nematospora gossypii*, the bios requirement of which is already known, is particularly interesting. It has been found that this fungus in addition to inositol above all needs biotin for growth in a synthetic medium. Aneurin in addition to these two factors increases the growth still more.

Finally, we have been able to prove that biotin and aneurin are also very important for the growth of higher plants. This can be shown with isolated pea embryos, which are cultivated under sterile conditions according to the method of Brown and Morris. Addition of biotin and aneurin (as well as oestron) stimulates growth. Since both biotin and aneurin are also present in the cotyledons, we may speak of a typical hormone action.

P. Fildes, F.R.S.: The first indications of the existence of nutritional factors necessary for the growth of bacteria were found in Johne's bacillus and in *B. influenzae* in 1912 and 1921, but from that time to 4 or 5 years ago, no biochemists being employed in this field, practically no advance was made. Vitamin-like activity had been ascribed to crude extracts, but the bacterial vitamin theory was considered unconvincing. The first preparation which has been thought worthy of this description was the "Sporogenes vitamin" described by Knight and myself in 1933. This preparation has not yet however been completely identified. In 1935 Knight separated a fraction which was regarded as a growth factor for *Staphylococcus* and in the present year has resolved this fraction into three parts, two being the pyrimidine and thiazole components of vitamin B_1 and the third being nicotinicamide, a component of Warburg's coenzyme and cozymase. Vitamin B_1 was also found last year by Tatum, Wood and Peterson to be a growth factor for propionic acid bacteria, while Lwoff and Lwoff showed that cozymase or Warburg's coenzyme was the "vitamin factor" of the Influenza bacillus. In addition to these identified growth factors Richardson has found that uracil is an essential factor in the anaerobic growth of *Staphylococcus*. It is now possible to grow *Staphylococcus* both aerobically and anaerobically on a diet, all constituents of which are known.

In 1924 in connexion with *B. influenzae* and its relatives we suggested that the necessity for a special component in the nutrients depended upon the failure of the organism to synthesize it. All bacteria examined, which do not require the growth factors hitherto mentioned, do synthesize these factors. The same applies to the essential amino acids. In the case of amino acids the bacteria which find them indispensable normally can be trained to synthesize them and these acids are then not necessary in the nutrients. Possibly the same will be found to apply to other growth factors. The nutritional requirements of bacteria depend upon their nutritional state, i.e. their powers of synthesis at the time of examination. The nutritional requirements of different species vary, hence their powers of synthesis. Thus *B. influenzae* cannot synthesize cozymase even from the components; *Staphylococcus* can synthesize the adenine from amino acids but not the nicotinicamide and can combine them; typhoid can synthesize both components from amino acids and combine them; an adapted typhoid can do the same from NH_3. The nutritional requirements of bacteria are an indication of the action of evolution as first indicated by Twort and Ingram in 1912. The parasitic bacteria have lost their power of synthesis owing to a persistent localization in an environment where their "factors" are preformed. This evolutionary process can be reversed in the test tube by deprivation of these factors. This hypothesis has already been advanced by Lwoff in the case of Protozoa. Attention is called to the increasing indications of a similarity in the nutrition of all cells, even bacterial and animal cells.

ANDRÉ LWOFF, *Institut Pasteur (Paris)*—*Haematin and Pyridine-nucleotide-phosphates considered as Growth Factors*

Some micro-organisms, bacteria: *Haemophilus influenzae* (Olsen, Fildes, A. and M. Lwoff), *Haemophilus canis* (Rivers), *Haemophilus ducreyi* (A. Lwoff and I. Pirosky) and Protozoa: *Strigomonas fasciculata, Strigomonas culicidarum, Strigomonas muscidarum, Leptomonas ctenocephali* (M. Lwoff) are unable to synthesize haematin. The addition of haematin to peptone solutions is necessary in order to obtain growth.

When approaching the limit of active dilutions the action of growth factors is quantitative. This was the origin of the method (A. Lwoff 1933) worked out in order to study *in vivo* the physiological function of these factors. The organisms are cultivated in presence of a small amount of growth factor so that this small amount is the only limiting factor of the development. Organisms grown under these conditions are referred to by the name of the growth factor followed by the sign −. The Q_{O_2} of "haematin+" *Strigomonas fasciculata* is 55, of "haematin−" 22. The $Q_{O_2}^{M}$ are respectively 1 and 19.

The addition of haematin produces an increase of oxygen consumption and a decrease of aerobic glycolysis. The increase of respiration is proportional to the amount of haematin when small quantities are added to the flagellates: 1/200 to 1/40μg. Similar results have been obtained with "haematin−" *Haemophilus influenzae* which is able to grow in a dilution of haematin approaching 10^{-9}. The number of molecules which are necessary for one flagellate has been estimated by different methods: (1) relation of growth to the amount of haemin, (2) increase of the Q_{O_2} with a given amount of haemin. The figures for one flagellate (dry weight, $6{\cdot}7 \times 10^{-6}\mu$g.) were respectively 746,000 and 520,000, i.e. 1/60,000 of the total weight.

The protohaemin and the protoporphyrin are the only active substances. A slight change in the structure of the molecule of these substances causes their inactivation as growth factors: deuterohaemin, haematohaemin, mesohaemin, pyrrohaemin, pheophorbidhaemin, iron salts, artificial peroxidases which are stabilized by means of colloids. Substances promoting growth have a definite influence on the respiratory processes. No mechanism of immediate cause and effect is claimed for this observed parallelism.

Cytochrome C alone is inactive but it is able to be used in the presence of haematin by *Strigomonas culicidarum*. These data will be discussed together with those concerning the growth factor V.

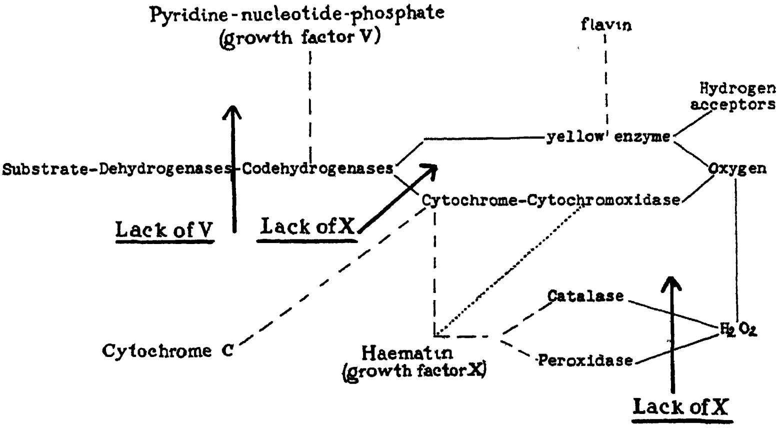
Growth factor V, which was discovered by Davis (1917), was recently identified with pyridine-nucleotide-phosphates (A. and M. Lwoff 1937*a*): Harden and Young's cozymase or Warburg's coenzyme (active at a dilution of about 6×10^{-8}); they act quantitatively on the growth of *H. parainfluenzae* and *H. influenzae*.

Experimentation on "V−" bacterias allows the study of the physiological function of pyridine-nucleotide-phosphates acting as growth factors.

The oxygen consumption of "V−" bacteria is increased about 10 times by pyridine-nucleotides; the aerobic and anaerobic glycolysis, about 20 times. The speed of reduction of methylene blue is increased, according to conditions, from 2 to 100 times. An incubation period of 90–150 sec. is necessary to allow the pyridine-nucleotides to act. When approaching the limit of dilution, the action of codehydrogenases on the speed of reduction of methylene blue and on respiration is quantitative as its action on growth.

The study of dehydrogenation shows the degree of importance of pyridine-nucleotides for various substrates. They are essential for dehydrogenation of glucose, pyruvate, fumarate, malate, ethylalcohol, asparagine, *d*- and *l*-valine, but not for *d*- and *l*-lactate and succinate.

On the other hand, it is possible to analyse the relation between pyridine-nucleotide-di- and triphosphates. Both of them may be used with pyruvate or hexosemonophosphate as substrate. The reaction pyridine-nucleotide-diphosphate ⇄ pyridine-nucleotide-triphosphate is therefore possible.



Further on it is possible to obtain three physiological types of bacteria:

(1) Bacteria with the normal amount of cozymase and of Warburg's coenzyme. This type is obtained in cultivating *H. parainfluenzae* with an excess of V factor. These "V+" bacteria oxidize glucose, hexosemonophosphate, pyruvate, fumarate at the same speed with or without added codehydrogenase.

(2) Bacteria with the normal amount of Warburg's coenzyme and without cozymase. This second type is obtained when "V−" bacteria are grown in peptone alone or peptone + pyruvate: these bacteria are able to oxidize glucose and hexosemonophosphate at the same speed with or without added codehydrogenase, but are unable to oxidize pyruvate and fumarate.

(3) Bacteria which are practically devoid of Warburg's coenzyme and cozymase. This type is obtained when cultivating "V−" bacteria in peptone + glucose, or peptone + hexosemonophosphate. This indicates that Warburg's coenzyme is used up in presence of its specific substrate only. The pyridine-nucleotide-triphosphate may exist in the bacteria in a combined state, being potentially active as coenzyme of the oxidation system of hexosemonophosphate but unable to undergo the conversion into pyridine-nucleotide-diphosphate.

We may now consider the growth factors in relation to the model of hydrogen mobilization due to the now classical researches of Wieland, Thunberg, Keilin and Warburg. The above schema, derived in part from Keilin's, shows clearly the relation existing between growth factors X and V and the prosthetic groups of catalysts and carriers. Growth factor V is a pyridine-nucleotide-phosphate; each of the di- or triphosphates may be converted into the other; both act in the bacteria as codehydrogenases. Growth factor X is haematin and enters in the constitution of Keilin's system: (1) directly in catalase and peroxidase, the prosthetic group of which is haematin: no direct evidence of this has yet been found; (2) after transformation in haemin C in the constitution of cytochrome C which is derived from protohaematin: *in vivo* observation and *in vitro* experiments of Keilin; (3) directly or after transformation in the constitution of Keilin's cytochromoxidase; this is only hypothetical. Cytochrome C may be used but does not allow the growth of the flagellates, probably because haematin is necessary as such and because the reaction haematin→heamin C is not reversible.

Conclusion—Two growth factors for micro-organisms are known at present in so far as their chemical constitution and physiological function are concerned; these are haematin and pyridine-nucleotide-phosphates. They do not act as true catalysts on multiplication. They are utilized in the synthesis of catalysts and carriers. The role of haemin and pyridine-nucleotide-phosphates considered as growth factors is none other than the role attributed to them by Keilin and Warburg.

B. C. J. G. KNIGHT—*The* Staphylococcus *and* Sporogenes *Growth Factors.*

The activity of potent concentrates of the growth factor required by *Staphylococcus aureus* (Hughes 1932; Knight 1935) has now been found to be due to nicotinic acid plus degradation products of aneurin, i.e. of vitamin B_1 (Knight 1937 *a*, *b*).

Nicotinic acid or its amide plus aneurin or certain compounds closely related to it, can completely replace the growth factor, enabling typical strains of the organism to be grown in a medium of known composition. The nicotinic acid and aneurin are only effective together; no growth occurs with either alone.

For maximum growth nicotinic acid (or amide) at approx. 10^{-6} M is needed, with aneurin at 10^{-8} M; a detectable effect is obtained with nicotinic acid at 10^{-8} M, and aneurin at 10^{-10} M.

Pyridine-3-nitrile cannot be used in place of nicotinic acid, but methyl nicotinate gives delayed growth, presumably due to slow hydrolysis of the inactive ester to the free active acid. The nicotinic acid is probably needed for the synthesis of cozymase by *Staphylococcus*, which is known to produce it.

Certain pairs of a pyrimidine plus a thiazole, both substituted respectively as in the pyrimidine and thiazole parts of the aneurin molecule, can be used by the organism instead of aneurin itself. Thus 4-amino-5-aminomethyl-2-methylpyrimidine plus 4-methyl-5-β-hydroxyethylthiazole can be used at the same molar concentrations as aneurin. Similarly 4-amino-5-thioformamidomethyl-2-methylpyrimidine functions in such a pair, and also thiochrome; but not 4-hydroxy-5-aminomethyl-2-methylpyrimidine, nor 4-hydroxy-5-hydroxymethyl-2-methylpyrimidine. On the other hand, 4-methylthiazole cannot be used in place of 4-methyl-5-β-hydroxyethylthiazole in any pair. And the corresponding aneurin which lacks the 5-β-hydroxyethyl group on the thiazole ring is inactive. Thiochrome alone has slight activity at about 1000 times the concentration at which aneurin is effective.

The two components of the growth factor are thus found to be compounds having wide biological importance, both cozymase and aneurin having recently been recognized as growth factors for other micro-organisms (cf. Tatum, Wood and Peterson 1936; Lwoff and Lwoff 1937*d*). Both these substances are synthesized by many bacteria which can grow in media not containing these substances, and may play a part in their metabolism.

The sporogenes growth factor is an essential nutrient for *Cl. sporogenes, botulinum* and *welchii* (Knight and Fildes 1933; Fildes 1935). This factor is characterized as an ether-soluble unsaturated hydroxy-acid of molecular weight about 200 and containing no N or S. The methyl ester is inactive and stable, the active free acid is unstable (Pappenheimer 1935).

Just as with nicotinic acid (or cozymase) and aneurin, it is found that a number of micro-organisms selected at random as able to grow in media not containing the sporogenes factor, do in fact synthesize this substance. It is a legitimate deduction that the sporogenes factor plays a part in the metabolism of those organisms which are able to synthesize it as well as in the metabolism of those organisms which cannot synthesize it, i.e. *Cl. sporogenes, botulinum* and *welchii* and which require it as an essential nutrient. The sporogenes factor may even play a part in animal metabolism; it is very widely distributed in vegetable and animal tissues of all kinds and is excreted in relatively large quantities in the urine of herbivorous animals.

G. M. Richardson*—*The Necessity for Nucleic Acid Components in the Anaerobic Growth of* Staphylococcus Aureus

The increasing evidence that the codehydrogenases and cophosphorylases of cellular metabolism include compounds of the class of nucleotides will direct attention again to the undiscovered metabolic function of nucleic acid. In the past, study of this function has been hindered by the experimental inability to demonstrate a need for nucleic components in growth or metabolism, presumably owing to the synthetic abilities of the cells.

If a need for nucleic acid components is to be demonstrated this should be sought, if anywhere, among organisms whose synthetic power has failed owing to habitual adaptation to a parasitic life. Thus it is reasonable that the indispensability of such components to growth has not been demonstrated except in the case of *Staphylococcus aureus*, and that the effect could only be observed under restricted conditions such as are found in anaerobic growth (Richardson 1936). Further work has shown that of eight strains examined anaerobically on a synthetic medium, none grew normally without added uracil (one of the pyrimidines of nucleic acid). Seven grew well with uracil as sole addition, but a supplementary addition of guanine improved the growth of two of these. The eighth strain required both uracil and guanine. *B. anthracis* grew only poorly anaerobically, but such growth as occurred was dependent on the presence of either uracil and guanine, or cytosine and guanine.

In discussing the possible function of uracil it is shown that known metabolically-active compounds which contain the pyrimidine ring (vitamin B_1, vitamin B_2, and adenine, a precursor of cophosphorylases) cannot be used to replace uracil, which therefore does not seem to function by conversion to these substances. It is hoped that the function of uracil may be discovered by studying the impairment of metabolic activity due to uracil deficiency, and it seems possible that this function will be identical with one of the functions of nucleic acid, in view of the connexion between the requirements for uracil and for other nucleic acid components. In this event its function may prove to be quite different from that of the nucleotide co-enzymes, since the ribose-phosphate linkages in these contrasted types of nucleotide seem to involve different C atoms (Levene and Stiller 1934).

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* Leverhulme Research Fellow, Bland-Sutton Institute of Pathology, Middlesex Hospital, London.

H. M. Sinclair (*Oxford*): Knight's observations on *Staphylococcus* have been extended to *Phycomyces*. Schopfer has very recently shown that 4-amino-5-aminomethyl-2-methylpyrimidine and 4-methyl-5-hydroxy-ethylthiazole can together replace vitamin B_1 as a growth factor for the mould. This I have confirmed, using synthetic compounds kindly supplied by Dr. Todd. Further, the 4-amino group in the pyrimidine compound is essential since the corresponding 4-hydroxy compound is inactive (Knight had found that this was true with *Staphylococcus*).

A neutral solution of vitamin B_1, autoclaved for 2 hours at 125° C., still acts as a growth factor for *Phycomyces*, although the compound is destroyed. The pyrimidine and thiazole compounds are still active after this treatment, even if done in presence of N/10 NaOH; H_2O_2, however, destroys the activity. This supports Schopfer's suggestion that his growth factor "MP" (which differs from vitamin B_1 in being very stable to heat and stable to alkali) consists of degradation products of the vitamin.

M. A. H. Tincker (*Royal Horticultural Society, Wisley*): During a series of experiments made to study the effect of applying extracts of yeast to the meristematic tissue of flowering plants a marked stimulation was observed on the rate of bacterial development in gelatin. The bacillus, described as *B. auxinophilum* by Jacobs (1935) showed this response to the petroleum ether-insoluble *J* fraction, prepared according to the methods of Fildes and Knight. Jacobs also observed similar stimulation in *ad hoc* tests. But as adequate nutrients were absent and as the minimum concentration at which stimulation took place was a high one (1 mg. per c.c.), compared with that necessary for *B. sporogenes*, the auxin compounds may well have been utilized in metabolism in a manner other than as accessory growth factors.

Again, when testing the addition of ketohydroxyoestrin and theelol to water cultures* of flowering plants, observations were made on the greatly accelerated development of bacteria occurring, despite all the usual precautions, in the culture solutions and on moistened cotton-wool plugs. Without these hormones no noticeable bacterial development took place. Ketohydroxoestrin, 0·0033 mg. per c.c., at *p*H 8·5 and similar quantities of theelol but at *p*H 7·2, caused this rapid bacterial development. The bacteria, probably of soil origin, were not identified; their requirements were thus unknown. The hormones may have proved a source of carbon.

* Water cultures contained, per litre, KNO_3 1 g., KH_2PO_4 0·3 g., K_2HPO_4 0·27 g., NaCl 0·5 g., $CaSO_42H_2O$ 0·5 g., $MgSO_47H_2O$ 0·5 g., Fe_2Cl_6 0·04 g.

The major part of the Wisley investigations concerns the effect of growth substances on flowering plants, particular attention has been paid to the initiation and acceleration of root formation to facilitate vegetative reproduction, but some results of anatomical interest have also been obtained.

Applied to young stems of tomato as a paste in lanolin at a concentration of 1 in 1000, small quantities (approximately 0·0005 g.) of β-indolyl acetic acid evoked rapid responses. Sixty-five hours after application Professor F. E. Weiss and the writer found that many epidermal, cortical, and pith cells were twice the size of comparable control cells. Radial walls freely developed in the cells of the epidermis, hypodermis and cortex of treated plants. Cell division, frequently repeated in scattered instances, occurred in the cells of the collenchyme, whilst meristematic activity was induced in the outer regions of the phloem tissue resulting in the production of root initials. The starch sheath concomitantly diminished. Lignification did not keep pace with the induced cell division in the xylem so that the new tissue was parenchymatous at this stage.

In woody cuttings the uptake from solutions of β-indolyl acetic acid or α-naphthalene acetic acid by cuttings results in the rapid formation of new parenchymatous tissue which often ruptures the outer layers of the stem. This "internal callus" attained in certain instances, as for example, *Tricuspidaria dependens*, a thickness of twenty cells in twenty-five days. A concentration of 1 part in 10,000 of water was used. Similarly with *Myrtus communis*, α-naphthalene acetic acid evoked in the natural season of quiescence rapid cell division to produce a cylinder of 15–20 cells in five weeks. The concentration used was 2 in 10,000. The region of stimulation was frequently limited to 1·5–2 cm. of the stem. Where the many roots emerged the number of cells formed in a few weeks was far greater than elsewhere.

Stimulation may occur in apical regions of the stems, as with *Buddleia alternifolia* where cell division and root formation has been produced amongst small young leaves from almost herbaceous stems. Illustrations are available in recent articles (Tincker 1936, 1937).

Possibly the natural seasonal excitation to cell division is a hormone-like reaction, caused by the downward transport of similar active compounds formed in young leaves.

REFERENCES

Fildes, P. 1935 *Brit. J. Exp. Path.* **16**, 309.
Hughes, T. P. 1932 *J. Bact.* **23**, 437.
Jacobs, S. E. 1935 *Ann. Appl. Biol.* **22**, 627.

Knight, B. C. J. G. 1935 *Brit. J. Exp. Path.* **16**, 315.
— 1937*a* *Biochem. J.* **31**, 731.
— 1937*b* *Biochem. J.* **31**, 966.
Knight, B. C. J. G. and Fildes, P. 1933 *Brit. J. Exp. Path.* **14**, 112.
Levene and Stiller 1934 *J. Biol. Chem.* **104**, 299.
References in A. and M. Lwoff 1937*a* *Proc. Roy. Soc.* B, **122**, 352–73.
Lwoff, A. and M. 1937*b* *C.R. Acad. Sci., Paris*, **204**, 1510.
— — 1937*c* *Ann. Inst. Pasteur*, **59**, 966.
— — 1937*d* *Proc. Roy. Soc.* B, **122**, 352, 360.
Pappenheimer, Jr., A. M. 1935 *Biochem. J.* **29**, 2057.
Richardson, G. M. 1936 *Biochem. J.* **30**, 2184.
Tatum, E. L., Wood, H. G. and Peterson, W. H. 1936 *Biochem. J.* **30**, 1898.
Tincker, M. A. H. 1935 *Ann. Appl. Biol.* **22**, 619.
— 1936 *J. Roy. Hort. Soc.* **61**, 380, 510.
— 1937 *Nature, Lond.*, **139**, 1104.

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On the Corpora Allata of Dipterous Insects

By E. T. Burtt

Entomology Department, Zoological Laboratory, Cambridge

(*Communicated by A. D. Imms, F.R.S.—Received* 1 *May* 1937)

It is well known from Nabert's extensive work (1913) on the corpora allata of the Insecta that these small organs are of general occurrence in that class of animals. In so far as the Diptera are concerned, this investigator gave little attention to that order, and only described the corpora allata in the adults of two species of *Tipula*. These organs have not so far been described in any of the Diptera Cyclorrhapha.

The corpora allata are attracting a good deal of attention at the present time because they are evidently the seat of hormone secretion. Thus, Wigglesworth (1934 and 1936) has shown on good evidence that, in the reduviid bug *Rhodnius prolixus*, they secrete the hormones which are intimately concerned with the processes of ecdysis and metamorphosis. Fraenkel (1935) also has demonstrated that a hormone induces pupation in the blow fly *Calliphora erythrocephala*. He gave evidence suggesting that this hormone is secreted in the region of the central nerve ganglia and is discharged into the blood. At the same time he did not locate the actual source of the hormone and remarked that "there exists no organ in the fly larva which can be homologized with the corpora allata of other insects". In the present paper endeavour is made to show that the homologues of these organs are present in the larva of *Calliphora* and that there is reason to regard them as the source of the hormone referred to Fraenkel.

METHODS

Dissections of third instar larvae of *Calliphora vomitoria* were made with the object of discovering whether the organ first described by Weismann in 1864 and often termed "Weismann's ring", or the "anneau du soutien", is in any way related to the corpora allata. Larvae of *Tipula* sp. and *Chironomus* sp. were also dissected and the relations of the corpora allata determined in these examples. In addition to dissections, whole preparations of these organs and their related parts were made. The finer details of the relations and the histology of the corpora allata were determined by means of serial sections. Material was fixed in aqueous Bouin's fluid and stained in Delafield's haematoxylin, iron-alum haematoxylin or with Mallory's triple stain. Chironomid larvae were punctured and fixed whole, but, with *Tipula* and *Calliphora* larvae, it was found more convenient to dissect out the parts required, before immersion in the fixative. In *Calliphora*, where comparisons were made between the histology of Weismann's ring in larvae of different ages, the length of time in the fixative and stain was, in each case, as nearly as possible the same.

CORPORA ALLATA IN LARVAE OF *CHIRONOMUS*

In the larva of *Chironomus* small bodies were described by Miall and Hammond (1900) under the name of paired ganglia. When examined in serial sections these organs are seen to be non-ganglionic structures, the histology of which is of the glandular type characteristic of corpora allata. Their relations with the stomatogastric system make it clear that these bodies are the organs in question. Holmgren (1904) recognized them as corpora allata and described the stomatogastric nervous system in more detail than Miall and Hammond. It will be noted on reference to fig. 1 that the recurrent nerve (*r*) bears a hypocerebral ganglion (*h*), just behind the level of the brain. It then divides into two branches which pass back to the stomachic ganglia (*pg*) at the sides of the proventriculus. The oesophageal ganglia (*og*) lie dorsally to the oesophagus, about half way between the brain and proventriculus. From each ganglion a nerve passes forward and meets the recurrent nerve of its own side; from this point a very fine nerve passes to the region of the cerebral commissure, and a broader strand of tissue passes forward and is attached to the trachea (*t*), but does not seem to make any nervous connexion with the brain at this point. All three cords, the recurrent nerve, oesophageal connective, and the strand to the trachea, meet at one point; and in this situation the corpus allatum (*ca*) of the side is located.

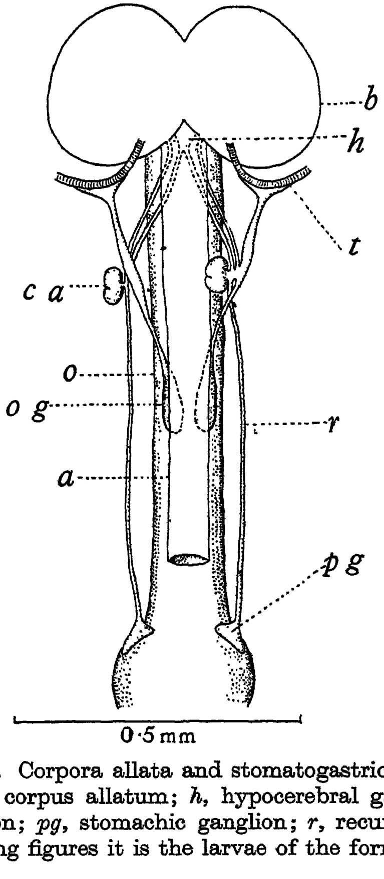

FIG. 1—*Chironomus* sp. Corpora allata and stomatogastric system in dorsal view. *a*, aorta; *b*, brain; *ca*, corpus allatum; *h*, hypocerebral ganglion; *o*, oesophagus; *og*, oesophageal ganglion; *pg*, stomachic ganglion; *r*, recurrent nerve. *t*, trachea. In this and the following figures it is the larvae of the forms named which is concerned.

The histology of the corpus allatum was described by Holmgren (1904). From fig. 3*a* it will be seen that the cells are few and rather variable in size. The cytoplasm is much vacuolated, the nuclei are large, and have large nucleoli and distinct chromosomes, recalling the condition in the nuclei of the salivary gland of this larva, only on a smaller scale. The organ is invested by a very fine connective tissue coat.

CORPORA ALLATA IN THE LARVAE OF *TIPULA*

The relations of the corpora allata in the larva of *Tipula* sp. are shown in fig. 2. The recurrent nerve (*r*) passes back from the frontal ganglion on the dorsal side of the oesophagus, under the brain. Immediately behind the brain it divides into two nerves which pass back to the proventriculus; there is no well-marked hypocerebral ganglion. At the dividing point of the recurrent nerve there arise two branches each of which passes upwards to a well-defined oesophageal ganglion (*og*), which is joined in its turn to the hind border of the brain. The corpora allata (*ca*) are compact oval bodies closely united to the oesophageal ganglia. Their histology is shown in fig. 3*b*; they possess a stout connective tissue coat and are composed of a mass of cells with well-defined nuclei, some of which show mitosis. The cytoplasm, in the particular specimen examined, was homogeneous and the cell boundaries were indistinct. The whole is rather similar to the condition described by Wigglesworth (1934) in *Rhodnius*.

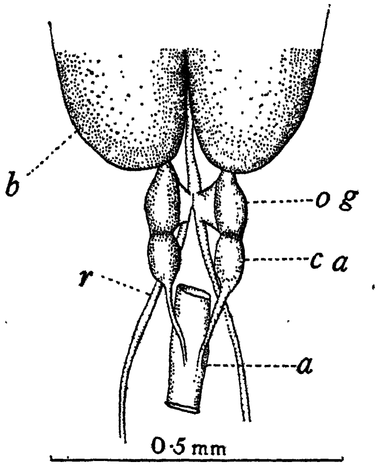

FIG. 2—*Tipula* sp. Corpora allata and stomatogastric system in dorsal view. *a*, aorta cut short; *b*, brain; *ca*, corpus allatum; *og*, oesophageal ganglion; *r*, recurrent nerve.

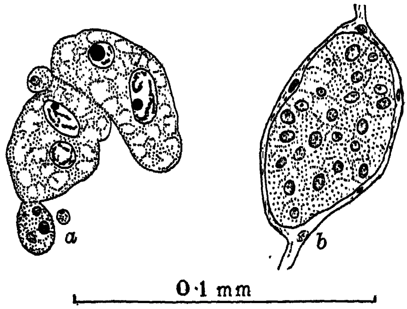

FIG. 3—Transverse sections of corpus allatum. *a* of *Chironomus* sp.; *b* of *Tipula* sp.

CORPORA ALLATA IN OTHER DIPTEROUS LARVAE

Structures which very probably represent the corpora allata have been described under different names in other nematocerous larvae. Imms (1908) in *Anopheles maculipennis* describes a supporting collar or "anneau de soutien" enveloping the aorta ventrally and laterally just behind the brain. It is composed of an irregular mass of cells which have no definite boundaries and are in some cases vacuolated. It is maintained in position by some fine muscle fibres arising from the head capsule and is attached by membranous strands to the oesophagus and the cerebral commissure. Anteriorly the organ is single but posteriorly it divides into two prolongations. This indication of a paired structure together with the type of cell structure points to this organ as representing the fused corpora allata. Dogiel (1877) in *Corethra* briefly mentions a similar mass of cells surrounding the aorta. Frew (1923) in *Forcipomyia piceus* describes under the name of Weismann's ring, a straight solid cellular rod lying dorsal to the heart. From each lobe of the brain, a small nerve mass arises, these fuse posteriorly and "Weismann's ring" lies behind their point of union. The anterior region of the ring gives indication of a paired structure. It seems reasonable to regard this organ as being the fused corpora allata, while the small nerve masses are in all probability the oesophageal ganglia.

WEISMANN'S RING IN CYCLORRHAPHOUS LARVAE

This organ, as previously mentioned, was discovered by Weismann in *Calliphora* in 1864. It was figured by Künckel d'Herculais (1875) in the larvae of Syrphidae, and Lowne (1890–1892) redescribed it in *Calliphora*. Pantel (1898) figured and briefly described its histology in the larva of the Tachinid *Thrixion* under the name of the "anneau de soutien"; he observed that the cells were sometimes vacuolated and that there were groups of small imaginal cells within the organ. Hewitt (1914) refers to the ring in *Musca domestica* and Thompson (1921) describes it in the larva of the Tachinid *Miltogramma punctatum* Meig. The latter author describes the ring as a pair of rods fused anteriorly above the aorta, and composed of cells with dense cytoplasm showing vacuoles. The organ is surrounded by a fine eosinophile capsule.

The relations of the ring and the stomatogastric nervous system were described by Lowne (1892). He states that the ring is innervated from a median ganglion situated immediately behind the commissure of the hemispheres and receiving several nerves from this region. The recurrent nerve passes back from the median ganglion to the single proventricular ganglion. Hewitt (1914) described a similar arrangement of the stomatogastric system in the larva of Musca, and Keilin (1917) describes and figures the condition in the larva of the Anthomyid *Phaonia*. His drawing is very similar to the one shortly to be described for *Calliphora* (fig. 4), except that he does not describe the median ganglion in the floor of the aorta.

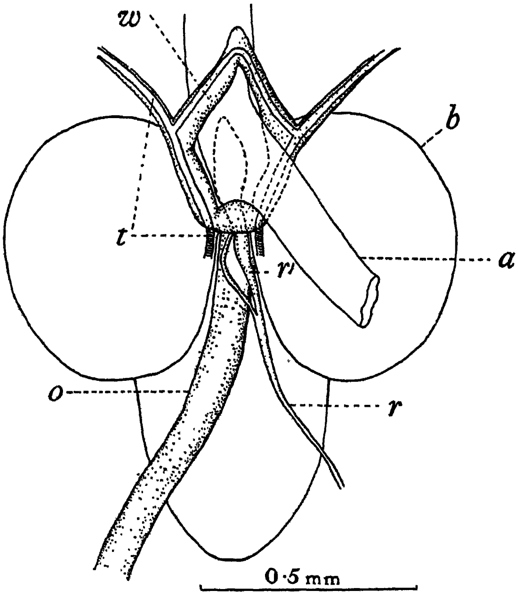

FIG. 4—*Calliphora vomitoria* L. Weismann's ring in dorsal view. *a*, aorta; *b*, brain; *o*, oesophagus; *r*, recurrent nerve; *r'*, branch of recurrent nerve to Weismann's ring; *t*, trachea; *w*, Weismann's ring.

WEISMANN'S RING IN *CALLIPHORA VOMITORIA* L.

The arrangement of the ring and the stomatogastric system in *Calliphora vomitoria* is shown in fig. 4, which is drawn from a dissection. The ring (*w*) is situated on the dorsal side of the cerebral lobes just above their point of union. It surrounds the aorta (*a*) which is attached to it dorsally and ventrally but is free at the sides. The ring is very readily found in dissection owing to the fact that two large tracheae (*t*), each of which originates on the inner aspect of the hemisphere of its side, pass forwards and outwards through the substance of the ring. After these tracheae have traversed about half the circumference of the ring they leave it and, just at this point, a short trachea unites those of the two sides; this trachea also traverses the ring. Thus, the ring is traversed by a tracheal tube throughout its circumference except just between the hemispheres. These tracheae pass through the ring but do not break up into any finer branches in its substance.

As regards the stomatogastric ganglia; there is a single ganglion on the proventriculus and from this the recurrent nerve (*r*) passes forward. Before it comes to the region of the cerebral commissure, it divides into two; the ventral branch passes under the brain, dorsal to the oesophagus, and so to the pharynx; the dorsal branch (*r*′) runs under the ventral part of the ring into a ganglion on the floor of the aorta. In fig. 4 this ganglion is covered by the ring and the aorta, but it is shown in sagittal section in fig. 5 (*m*). This ganglion appears to correspond with the median ganglion of Lowne (1892). Its shape is not sharply defined but it can be recognized by the presence within it of triangular nerve cells (fig. 5 *nc*) and, further, it receives a pair of nerves (*n*) on each side from the cerebral commissure. The ventral portion of Weismann's ring is in intimate contact with the median ganglion.

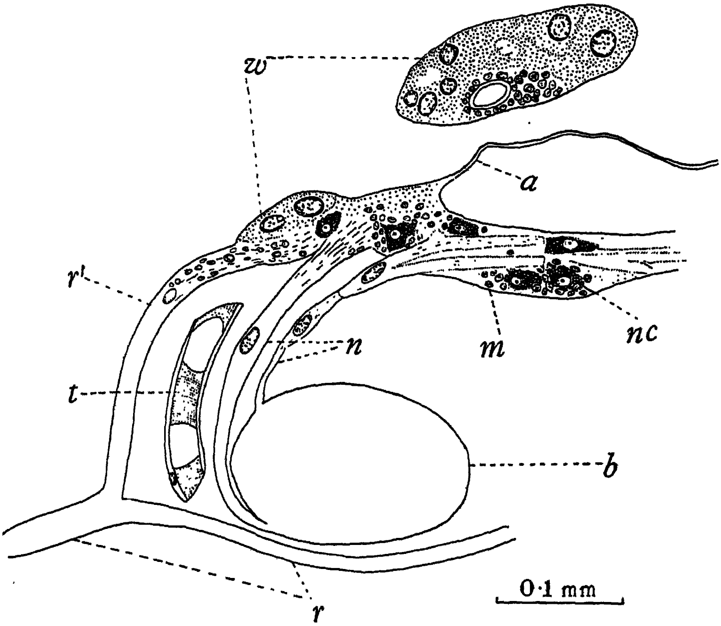

FIG. 5—*Calliphora vomitoria* L. Median sagittal section between the cerebral hemispheres. *a*, aorta; *b*, brain; *m*, median ganglion; *n*, nerves to median ganglion; *nc*, nerve cells; *r*, recurrent nerve; *r*′, branch of recurrent nerve to Weismann's ring; *t*, trachea; *w*, Weismann's ring.

DISCUSSION

It will be evident from the foregoing account that the corpora allata show marked differences in general configuration among larvae of several of the Diptera Nematocera. Thus in *Chironomus* (fig. 1) they are distinct organs relatively widely separated from each other and the oesophageal ganglia. In contrast to this more or less generalized condition, that found in *Tipula* (fig. 2) is noteworthy: here the shortening of the nerve connectives has caused the oesophageal ganglia and corpora allata to come to lie just behind the brain. Further, the corpora allata and oesophageal ganglia are closely connected together. In *Anopheles* the corpora allata are further concentrated and fused. According to Imms (1908), they are fused anteriorly and embrace the aorta and, posteriorly, they show a paired structure. In the account given by Frew (1923) they are also highly modified in *Forcipomyia*. *Anopheles* seems to be transitional between the condition observed in *Chironomus* and *Tipula*, and that of the Diptera Cyclorrhapha, where the corpora allata become fused into an annular structure and lose all trace of paired origin. Presumably then, the median ganglion of Lowne (1892) will represent the fused oesophageal ganglia, as its direct innervation from the brain would suggest. This series makes it fairly clear that Weismann's ring represents the highly modified corpora allata of the lower Diptera and other insects. In this connexion, it is interesting to note that Pratt (1900), in an account of the embryology of the Hippoboscid *Melophagus*, refers to the origin of ganglia allata (an earlier term for corpora allata) in this insect. He describes them as a pair of masses of nervous tissue which agreed with the position of origin of the ganglia allata found by Heymons (1895) in Orthoptera. They moved up dorsally and fused anteriorly; Pratt did not follow their development into the full grown larva.

Changes in Weismann's Ring Preceding Pupation

If the ring be the corpora allata we should expect its cells to be of a glandular type. A longitudinal section is shown in figs. 6*a* and *b*. The organ is invested by a fine connective tissue coat continuous with that of the aorta. The cells composing it are of three types:

(1) A group of small darkly staining imaginal cells with little cytoplasm (*ic*); these are situated around the tracheae which traverse the ring.

(2) A few cells (*nc*) of nervous type scattered through the ventro-lateral portion of the ring.

(3) Large cells with large nuclei, rather indistinct cell boundaries and

often showing vacuoles in the cytoplasm. These apparently are to be regarded as being secretory cells (*sc*).

In view of the marked changes found by Wigglesworth (1934), in the histology of the corpora allata after hormone secretion, it is natural to enquire whether any analogous changes can be seen in connexion with the cells of Weismann's ring in *Calliphora.* Lowne (1892) observed the external changes in the shape of the ring which take place in the larva, after it has ceased feeding, and is resting prior to pupation. He states that the ring increases in size and comes to lie more nearly horizontally. This increase in size is due, however, to the growth of the imaginal bud cells (*ic*) in the ring during this period, in common with those of the neighbouring eye and limb rudiments.

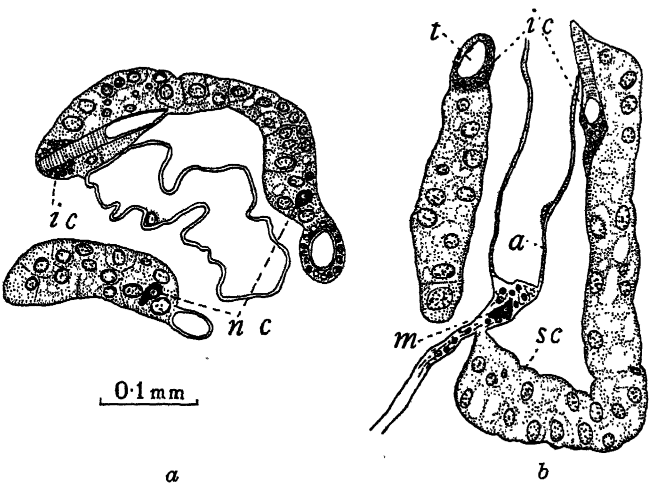

FIG. 6—*Calliphora vomitoria* L. *a*, longitudinal section of Weismann's ring in larva at end of period of feeding; *b*, the same about 12 hr. before pupation; *a*, aorta; *ic*, imaginal bud cells; *m*, median ganglion; *nc*, nerve cells; *sc*, secretory cells; *t*, trachea.

As regards the secretory cells of the ring, histological changes are observable during the period prior to pupation. A group of larvae were reared together at 20° C. so that they should all be as nearly as possible at the same stage in development. Larvae were examined at intervals during the 3–4 day period intervening between the cessation of feeding and the onset of pupation. The "critical period" was the same for *Calliphora vomitoria* as that found by Fraenkel (1935) in *C. erythrocephala*, i.e. 16 hr. before pupation at 20° C.

Fig. 6*a* shows the ring in a larva that has ceased feeding, and is about 3 days before pupation; fig. 6*b* shows the ring in a larva about 12 hr.

before pupation. It will be seen that in the earlier stage small vacuoles are present and the cell boundaries can be made out. In the later stage (fig. 6*b*) the vacuoles are much larger and have become confluent and the cell boundaries are less evident. Similar changes were found by Wigglesworth (1934) in the corpora allata of *Rhodnius* before the last nymphal ecdysis. In the series obtained of *Calliphora* it was not possible to detect a change sharply restricted to a short period, such as might be expected to coincide with the release of the hormone.

The structure of the ring cells together with the changes that they show agree with the view that this organ secretes the hormone discovered by Fraenkel (1935). In a later communication it is hoped to bring forward evidence on this matter based on experiments involving extirpation of Weismann's ring.

I should like to take this opportunity of thanking Dr. A. D. Imms, F.R.S., for his kind help and criticism.

SUMMARY

The corpora allata are described in *Chironomus* and *Tipula* and their occurrence in other Diptera Nematocera briefly reviewed.

Weismann's ring of cyclorrhaphous larvae is described in *Calliphora* and reasons are given for concluding that this organ represents the modified and fused corpora allata.

Weismann's ring is regarded as the probable seat of origin of the hormone discovered by Fraenkel which controls pupation in *Calliphora*.

REFERENCES

Dogiel, J. 1877 *Mém. Acad. Sci. St.-Pétersb.* 7 Sér. t. **24**, No. 10.
Fraenkel, G. 1935 *Proc. Roy. Soc.* B, **118**, 1.
Frew, J. G. H. 1923 *Ann. Appl. Biol.* **10**, 432.
Hewitt, C. G. 1914 "The House Fly." Cambridge.
Heymons, R. 1895 "Embryonalentwicklung von Dermapteren u. Orthopteren." Jena.
Holmgren, N. 1904 *Z. wiss. Zool.* **76**, 456.
Imms, A. D. 1908 *Parasitology*, **1**, 114.
Keilin, D. 1917 *Parasitology*, **9**, 367.
Künckel d'Herculais, J. 1875 "Recherches sur l'organisation et le développement des Volucelles." Paris.
Lowne, B. T. 1890–92 "The Blow Fly." London.
Miall, L. C. and Hammond, A. R. 1900 "The Harlequin Fly." Oxford.
Nabert, A. 1913 *Z. wiss. Zool.* **104**, 328.

Pantel, J. 1898 *Cellule*, **15**, 171.
Pratt, H. S. 1900 *Proc. Boston Soc. Nat. Hist.* **29**, 241.
Thompson, W. R. 1921 *Bull. Biol.* **54**, 314.
Weismann, A. 1864 *Z. wiss. Zool.* **14**, 211.
Wigglesworth, V. B. 1934 *Quart. J. Micr. Sci.* **77**, 191.
— 1936 *Quart. J. Micr. Sci.* **79**, 91.

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The Mucification of the Vaginal Epithelium in Rodents

By M. Klein*

From the National Institute for Medical Research, London

(*Communicated by A. S. Parkes, F.R.S.—Received 3 May* 1937)

[Plates 1, 2]

Introduction

The mucosa of the vagina of small rodents undergoes during pregnancy and in some cases during pseudo-pregnancy a special transformation which results in complete mucification of the epithelium. This mucification was recorded in observations as far back as 1890 by Morau (1889), Lataste (1892), Retterer (1892*a*, *b*), and the descriptions of these authors caused important discussions on the structure of the vaginal epithelium. The study of this mucification was resumed when the vaginal cycle was considered in relation to the ovarian cycle (Long and Evans 1922; Selle 1922; Courrier 1924; Kelly 1929), and some of its cytological features were described by Florey (1932). The causation of the vaginal mucification was not thoroughly investigated until about 1930, when a number of workers postulated that the reaction was a specific and convenient test for the *corpus luteum* hormone (Harris and Newman 1931; Hisaw, Meyer and Weichert 1928; Wiesner and Patel 1929; Clauberg 1933). It was shown shortly afterwards however, that the mucification obtained with injections of *corpus luteum* extracts was probably dependent upon small amounts of oestrone contained in those extracts (Robson 1931; Meyer and Allen 1933; Fels 1934). Desclin

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* Of the Institut d'Histologie, Strasbourg.

(1934) and Allen and Meyer (1935) later demonstrated that the effect seemed to be due to the inhibition of oestrone by progestin. I have shown (Klein 1935*a*) that the maintenance of the mucification during pregnancy is due to the inhibition of the ovarian cycle by the placenta. Recently Selye, Browne and Collip (1936*a*) showed that combined administration of crystalline oestrone and progesterone to the adult ovariectomized rat produces mucification of the vaginal epithelium, while progesterone injected to the normally cyclic female rat does not (1936*b*). It is clear from this short survey that the real causes of vaginal mucification are not yet satisfactorily known. In the present paper I give a report of experiments carried out on the golden hamster.

MATERIAL AND TECHNIQUE

The experiments were carried out on the golden hamster (*Cricetus* (*Mesocricetus*) *auratus* Waterhouse). No experimental work has been done so far on this species. Zoological notes on its breeding and growth have been published by Bruce and Hindle (1934), and a study of the ovarian cycle will be published in the near future by Dr. Ruth Deanesly. The animals at my disposal were bred from a stock kept at the National Institute for Medical Research, Hampstead.

The hamsters used for these experiments were adult females ovariectomized at least 2 months previously. They were injected intramuscularly for 5 days with arachis oil solutions of crystalline hormones; they were killed the day following the last injection.

The hormones injected were (1) oestrone, and (2) crystalline progesterone, kindly supplied by Schering-Kahlbaum A.G. The physiological potency of the latter was tested on the endometrium of the immature rabbit by the method of McPhail (1934).

All tissues for histological examination were fixed in Bouin's fluid and the slides stained with haematoxylin and eosin or with mucicarmine and methanyl yellow.

EXPERIMENTAL RESULTS

Structure of the Vaginal Mucosa during Pregnancy—Before recording the results obtained with injections of female sex hormones, we must describe the mucification occurring in the upper and middle parts of the vagina of the golden hamster during pregnancy. The lower part does not mucify or undergo cyclic changes of any kind. The vaginal epithelium is a stratified or a pseudostratified squamous epithelium except in pregnancy or pseudopregnancy, when it becomes simple and columnar and mucified. It is then constituted as a layer of tall cylindrical cells completely filled with a mucous substance which stains a dark red with mucicarmine. Beneath these cylindrical cells, there is a layer of basal cells which are so flat that only the nuclei are visible below the mucified cells (fig. 1, Plate 1). These latter are not of the goblet cell type, but are rather similar to the surface cells of the gastric mucosa. We shall describe elsewhere the cytological features of these mucous cells; but it should be emphasized that the type of mucification occurring in the hamster makes it particularly favourable for the study of vaginal mucification in general.

Structure of the Mucosa after Ovariectomy (fig. 2, Plate 1)—Two months after ovariectomy the epithelium is cubical or cylindrical and bistratified, and there are gland-like invaginations of the epithelium into the connective tissue. Here and there small mucous droplets may be seen in the apical part near the free border of the cells. These rare droplets may be observed up to 5 months after ovariectomy, and in the lumen of the gland-like invaginations there often remain clots of substance staining with mucicarmine. The reason for this very discrete mucous secretion, shown only by mucicarmine, is not yet quite clear.

Effect of Injections of Oestrone alone—The effect of oestrone on the vaginal epithelium varies with the amount injected.

Total amount 2·5γ oestrone. The cells grow higher and become pseudostratified; the droplets of mucus scattered in the apical part of the cells are more abundant than after ovariectomy (fig. 3, Plate 1).

Total amount 5γ oestrone. The epithelium is stratified squamous, without mucus, and with here and there a very slight cornification (fig. 4, Plate 1).

Total amount 10γ or more. The epithelium is squamous stratified with complete cornification of the superficial layers (fig. 5, Plate 2). The picture is the usual one for oestrus in other small rodents, but it must be emphasized that in the hamster the oestrous appearance of the upper vagina during the normal cycle is different (Deanesly, in press).

Effect of Progesterone alone—Crystalline progesterone, injected in amounts of 0·5 or 1 mg. (one International Unit), does not cause the slightest modification of the vagina of the castrate (fig. 6, Plate 2). The cells remain low and bistratified and occasional mucous droplets are observed as after ovariectomy (compare fig. 6 with fig. 21).

Effect of Progesterone after Oestrone—2·5γ oestrone was injected first over 5 days, followed by 0·5 or 1 mg. progesterone. The epithelium is pseudostratified and does not contain any mucus, but shows a transmigration of leucocytes, which are also present in the lumen in fairly large numbers (fig. 7, Plate 2). This picture is very like the classical picture of metoestrus in other rodents.

Effect of Progesterone together with Oestrone—5γ oestrone was injected *simultaneously* with 0·5 or 1 mg. progesterone. The epithelial cells are high columnar, unilayered and completely filled with mucus which stains a dark red with mucicarmine. Under this layer of mucified cells there is a row of nuclei of the basal layer (fig. 8, Plate 2). The picture is identical with that of the mucification observed during pregnancy or pseudopregnancy (compare fig. 8 with fig. 1).

It is clear, therefore, that oestrone alone causes a stratification or a cornification of the epithelium, progesterone alone does not alter the epithelium at all, progesterone after oestrone gives a pseudostratification with leucocytes, while progesterone together with oestrone produces a tall columnar mucified epithelium such as is seen during pregnancy.

It remains to discuss these results and compare the interpretation they suggest with those put forward by other authors.

DISCUSSION

As mentioned above, a very small number of scattered mucous droplets may be found in the apical part of epithelial cells in the spayed animal even several months after ovariectomy. Courrier (1924) had observed a layer of mucified cells in the vagina of the immature and of the ovariectomized guinea-pig, as well as in the pregnant one. Thus he supposed that mucification was a sign of ovarian quiescence. We cannot agree with this interpretation, so far as the golden hamster is concerned. The amount of mucus observed after ovariectomy is exceedingly small and is in no way comparable with the mucification occurring during pregnancy.

Progesterone alone is not able to cause the slightest mucification, and this reaction of the vagina is therefore useless as a test for *corpus luteum* hormone.

Oestrone alone affects the epithelium in various ways according to the amount injected. Very slight amounts cause an increase in the occasional droplets, together with a pseudostratification. But in this case also the picture is far from being the same as during pregnancy, so that full mucification is not merely the effect of small amounts of oestrone.

Progesterone together with oestrone causes the epithelium to assume full mucification identical with that seen during pregnancy. In consequence, full mucification is a sign of simultaneous action of progesterone and oestrone. This interpretation is similar to those suggested by Desclin (1934), Allen and Meyer (1935), Selye, Browne and Collip (1936*a*). Desclin and Allen and Meyer, after combined injections of oestrone and progestin, postulated that vaginal mucification is due to inhibition of oestrone by progestin and is a manifestation of the antagonism between the two ovarian hormones, which had previously been demonstrated in different experiments by Courrier and his co-workers (cf. the general review by Courrier 1935).* It now seems preferable to consider that mucification is produced experimentally by a well balanced synergism and equilibrium between oestrone and progesterone; previous experiments on the rat (Klein 1935*b*) and others recently carried out on the golden hamster, suggest that a similar mechanism is responsible for the mucification occurring during normal pregnancy.

I should like to express my very best thanks to Sir Henry Dale, F.R.S., and the Medical Research Council for the hospitality of the National Institute for Medical Research, London, 1935–6.

It is with pleasure that I acknowledge my debt of thanks to Dr. A. S. Parkes, F.R.S., and Dr. Ruth Deanesly for constant and helpful advice during the course of these experiments. My thanks are also due to Dr. R. K. Callow for preparing the solutions injected. I am indebted to Dr. Schoeller of Schering-Kahlbaum A.G., who generously supplied synthetic crystalline progesterone.

SUMMARY

Ovariectomized golden hamsters were injected with crystalline ovarian hormones. Whilst oestrone alone, progesterone alone, or progesterone following oestrone were not able to cause mucification of the epithelial cells of the vagina, progesterone together with oestrone produced high columnar cells completely filled with mucus, a picture identical with that observed during pregnancy.

The appearance and maintenance of full mucification of the vaginal epithelium seem therefore to be due (at least in the golden hamster) to the simultaneous action of balanced amounts of oestrone and progesterone.

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* Courrier and Cohen-Solal have just published a note (1937) describing the effect on the vaginal smear in the rat of simultaneous injections of oestrone and progesterone.

REFERENCES

Allen, W. M. and Meyer, R. K. 1935 *Anat. Rec.* **61**, 427–39.
Bruce, H. M. and Hindle, E. 1934 *Proc. Zool. Soc. Lond.* pp. 361–6.
Clauberg, C. 1933 "Die weiblichen Sexualhormone", 1st ed. p. 141. Berlin: Springer.
Courrier, R. 1924 *Arch. Biol.* **34**, 369–477.
— 1935 *Bull. Histol. Tech. micr.* **12**, 261–8.
Courrier, R. and Cohen-Solal, G. 1937 *C.R. Soc. Biol., Paris*, **124**, 961–4.
Deanesly, R. *Proc. Zool. Soc. Lond.* (in press)
Desclin, L. 1934 *Arch. int. Med. exp.* **9**, 5–19.
Fels, E. 1934 *Arch. Gynaek.* **158**, 364–92.
Florey, H. 1932 *Brit. J. Exp. Path.* **13**, 323–7.
Harris, R. G. and Newman, D. M. 1931 *Science*, **74**, 182.
Hisaw, F. L., Meyer, R. K. and Weichert, C. K. 1928 *Proc. Soc. Exp. Biol., N.Y.*, **25**, 754–6.
Kelly, G. L. 1929 *Amer. J. Anat.* **43**, 247–87.
Klein, M. 1935*a* *C.R. Soc. Biol., Paris*, **119**, 577–8.
— 1935*b* *Arch. Anat. Micr.* **31**, 397–416.
Lataste, F. 1892 *C.R. Soc. Biol., Paris*, **44**, 765–9.
Long, J. A. and Evans, H. M. 1922 *Mem. Univ. Calif.* **6**. Univ. Calif. Press, Berkeley, Calif.
McPhail, M. K. 1934 *J. Physiol.* **83**, 145–56.
Meyer, R. K. and Allen, W. M. 1933 *Anat. Rec.* **56**, 321–43.
Morau, H. 1889 *J. Anat., Paris*, **25**, 277–97.
Retterer, E. 1892*a* *C.R. Soc. Biol., Paris*, **44**, 101–7.
— 1892*b* *C.R. Soc. Biol., Paris*, **44**, 566–8.
Robson, J. M. 1931 *J. Physiol.* **71**, iii.
Selle, R. M. 1922 *Amer. J. Anat.* **30**, 429–49.
Selye, H., Browne, J. S. L. and Collip, J. B. 1936*a* *Proc. Soc. Exp. Biol., N.Y.*, **34**, 198–200.
— — — 1936*b* *Proc. Soc. Exp. Biol., N.Y.*, **34**, 472–4.
Wiesner, B. P. and Patel, J. S. 1929 *Nature, Lond.*, **123**, 449.

DESCRIPTION OF PLATES

(All illustrations are of the vagina of the golden hamster and are magnified 280 diams.)

Plate 1

FIG. 1—Normal 8th day of pregnancy. Epithelium unilayered, cells high columnar, all mucified.

FIG. 2—Castrate. Epithelium bistratified, cells low, scarce mucous droplets here and there.

FIG. 3—Castrate, injected with 2·5γ oestrone. Slight pseudostratification, cells high, mucous droplets in some.

FIG. 4—Castrate, injected with 5γ oestrone. Epithelium stratified, cells squamous.

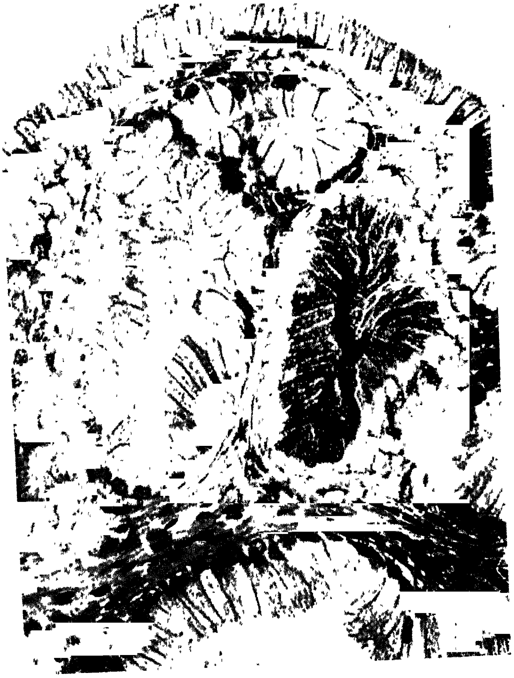

FIG. 1

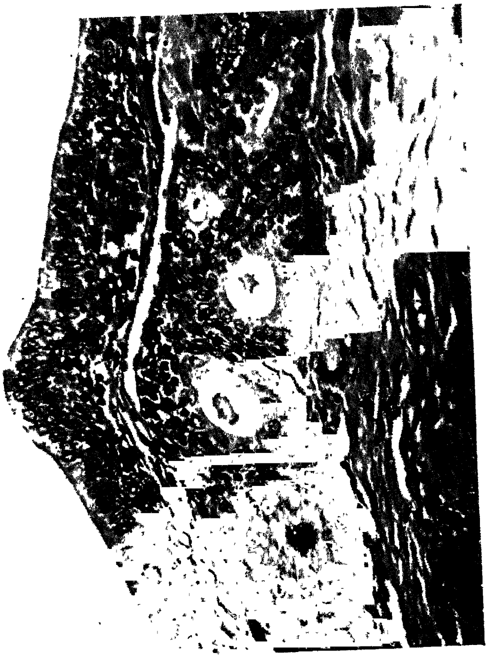

FIG. 2

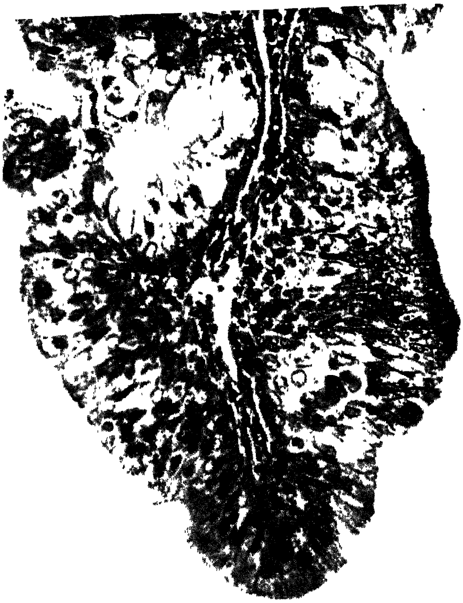

FIG. 3

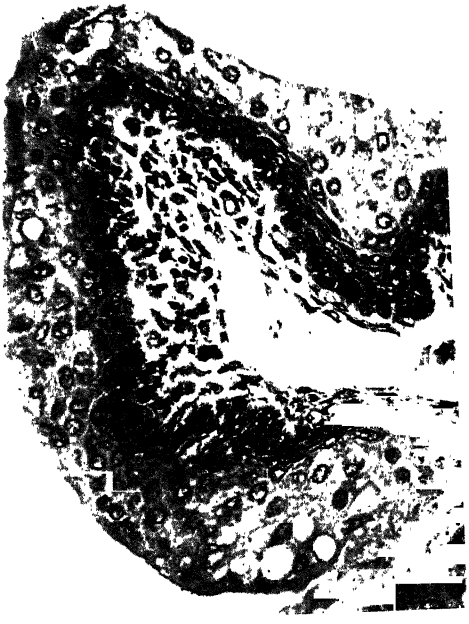

FIG. 4

(*Facing p.* 28)

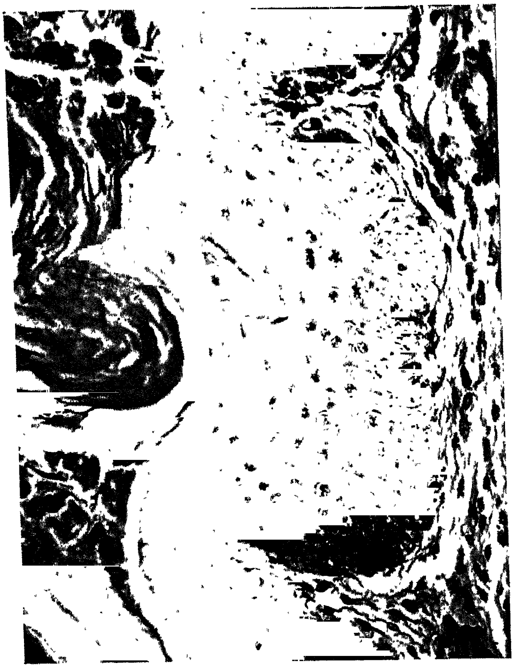

Fig. 5

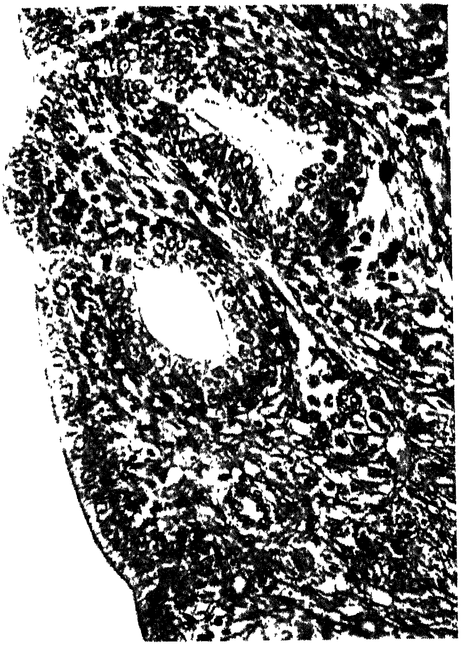

Fig. 6

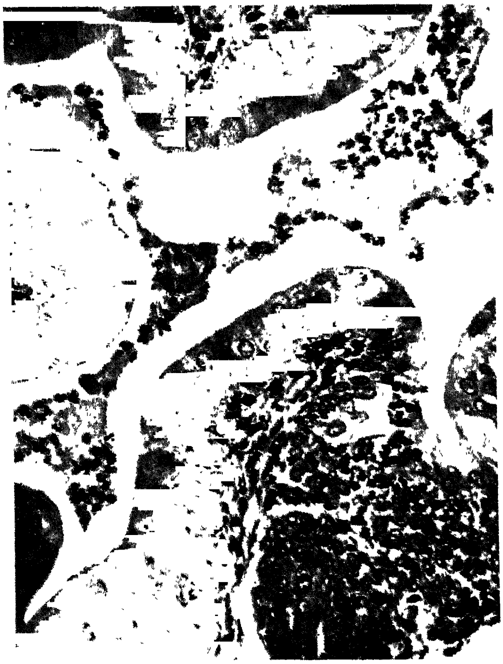

Fig. 7

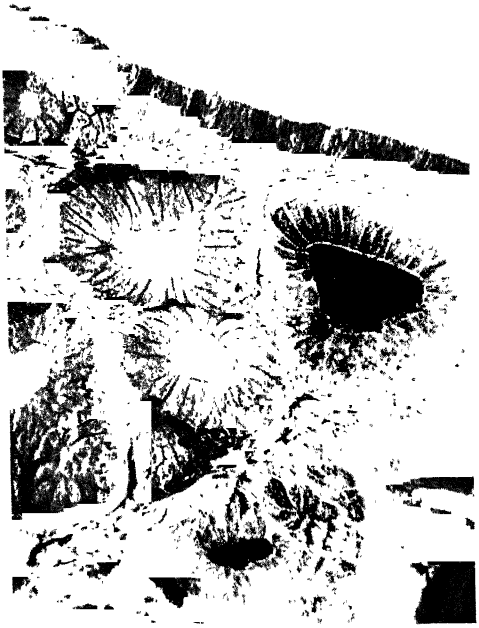

Fig. 8

Plate 2

Fig. 5—Castrate, injected with 10γ oestrone. Epithelium squamous stratified; complete cornification of the superficial layers; cornified cells in the lumen.

Fig. 6—Castrate; injected with 1 mg. of progesterone. Epithelium bistratified, cells low, very scarce mucous droplets. Compare with fig. 2.

Fig. 7—Castrate; injected with 2·5γ oestrone, *followed* by 1 mg. progesterone. Epithelium bi- or pseudostratified, cells fairly high. Numerous leucocytes in the mucosa and in the lumen.

Fig. 8—Castrate; injected *simultaneously* with 5γ oestrone and 1 mg. progesterone. Epithelium unilayered, cells high columnar, filled up with mucus. Compare with fig. 1.

612.741.4

The Thermoelastic Properties of Muscle and their Molecular Interpretation

By Kurt H. Meyer

Laboratoires de chimie organique et inorganique de l'université, Genève,

and L. E. R. Picken

Zoological Laboratory, Cambridge

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[Plate 3]

1—Introduction

As is the case with all physical properties, the mechanical properties of an object may be referred ultimately to the arrangement of its atoms and the forces between them. It is therefore possible under certain conditions to draw conclusions about the fine structure of an object from its mechanical behaviour.

The analysis of thermoelastic properties in particular has led, in a number of cases, to definite conclusions about molecular structure, and it has been the object of the work described here to apply the same method to the study of muscle.

The Thermodynamic Treatment of Thermoelastic Properties

The method is to determine the changes in internal energy and entropy (together constituting the free energy change) which result when a given object is reversibly deformed. The interpretation of the results obtained is based on the relation between thermodynamics and the kinetic theory of heat.

If a stretched object is in equilibrium with the deforming force, it may (under certain conditions) perform work reversibly, and it is then permissible to apply to this system the equations of thermodynamics. From simple considerations it can be shown that the temperature coefficient of the force K, exerted by a deformed (e.g. stretched) object in equilibrium at constant length, can be used immediately to determine dE and $-TdS$, whose sum constitutes the free energy increment for an infinitely small deformation. It can be shown namely that

$$K = \left(\frac{\partial E}{\partial l}\right)_T + T\left(\frac{\partial K}{\partial T}\right)_l \tag{1}$$

and

$$\left(\frac{\partial K}{\partial T}\right)_l = -\left(\frac{\partial S}{\partial l}\right)_T, \tag{2}$$

where $K =$ elastic force, $E =$ internal energy, $l =$ length, $T =$ absolute temperature and $S =$ entropy. (The derivation and detailed discussion of these equations will be found in Meyer and Ferri 1936 and Meyer, Lotmar and Pankow 1936.)

Over a small range of temperature, K may be treated as a linear function of T (the curve $K = f(T)$ is replaced by its tangent). The equation of this straight line is then

$$K = a + bT, \tag{3}$$

where $a = \left(\frac{\partial E}{\partial l}\right)_T$ and $b = \left(\frac{\partial K}{\partial T}\right)_l$.

From this result the following conclusions may be drawn:

(1) If in a given range of temperature, K is found to increase proportionally with the absolute temperature, $a = 0$, and an isothermal stretch or contraction takes place without change in E. If a is positive, E diminishes during an isothermal contraction; conversely, if a is negative, E increases during contraction.

(2) If the force, K, is found to be independent of the temperature, S remains unchanged during isothermal stretching or contraction. When K increases with rising temperature, S increases during contraction; similarly,

if K diminishes as the temperature rises, S diminishes during isothermal contraction.

The Molecular Interpretation of the Thermodynamic Results

In terms of the mechanical theory of heat, $\left(\frac{\partial E}{\partial l}\right)_T$ represents the change in the potential energy of molecules or parts of molecules, which takes place during an isothermal change in length.

(*a*) If $\left(\frac{\partial E}{\partial l}\right)_T$ is positive, saturation of forces of attraction occurs during the process of contraction. In the special case of the elastic return of a deformed body, this means that atoms, which were displaced by stretching from positions of minimal potential energy (potential troughs), return to their former condition.

(*b*) When $\left(\frac{\partial E}{\partial l}\right)_T = 0$, the work done during contraction results from a rearrangement of the kinetic units (atoms or groups of atoms); in other words, thermal agitation alone is responsible for the contraction.

(*c*) In the case in which $\left(\frac{\partial E}{\partial l}\right)_T$ is negative (i.e. dE is positive during an isothermal contraction), the tendency for a less ordered arrangement of the kinetic units to supervene is so great that more heat is absorbed than is realized as work. The nature of this excess heat will be discussed at the end of this section.

The entropy of the system is related to the thermodynamic probability by the equation

$$S = R \ln \mathrm{P}.$$

P provides a measure of the possibilities of movement and rearrangement open to the kinetic units. If, therefore, the entropy, S, increases on stretching the object, this means that deformation provokes a transformation from a more ordered to a less ordered condition—the sphere of movement of the kinetic units is increased.

Conversely, if S diminishes on stretching (as happens in the case of rubber and other rubber-like high polymers at moderate elongation), the possibilities of rearrangement and of movement of the units are limited by the stretch—the system becomes more orderly. This process can consist simply in a rearrangement of the units without any change in internal energy; it may, however, be accompanied by a reduction in internal energy, if crystalline regions are formed with liberation of heat of crystallization.

A number of substances are known which are characterized by a low modulus of elasticity and high reversible extensibility. The prototype of all these is rubber, but similar high extensibility is found in substances of quite different chemical composition, e.g. elastic sulphur, polyphosphonitrilic chloride, elastic fibres, etc. In all these the elastic force at moderate elongation increases proportionally with the absolute temperature (or increases more rapidly, i.e. a is negative).

The contractile mechanism in these substances is therefore different in principle from that in a deformed crystal or glass, whose atoms are displaced from their potential troughs by stretching. In rubber-like substances only the arrangement of the atoms is changed on stretching (in a given range of temperature and elongation), becoming more regular and less probable as a result of orientation. Thermal agitation causes the system to return to the less ordered and more probable state, as soon as the deforming force is removed.

Contraction depends therefore on the tendency of the ordered system to pass into a less-ordered condition. Such disorientation is not possible (in the case of these substances) without a macroscopic contraction of the stretched object. This fact leads one to conclude that the groups of atoms in the orientated condition do not become disorientated in the same way as do orientated permanent dipoles in a liquid, when the orientating field disappears. The atoms or groups of atoms must therefore be bound together by strong bonds in the direction of stretch. This means that the rubber-like substances are built up of chain molecules. The rearrangement on stretching consists therefore in a straightening out and parallel orientation of the chains.

It is thus possible to make a statement about the kind of molecules present in an object from a knowledge of its thermoelastic behaviour alone.

The straightening out of the chains on stretching leads further to the conclusion that the atoms or groups of atoms belonging to neighbouring chains are able to slide over each other like the molecules in a viscous liquid. On the other hand, each atom or group is firmly bound to two neighbours. We can say therefore that an atom or group is in one dimension, "solid", i.e. is held by bonds such as occur in a solid body and to which the great internal friction ($\eta > 10^{11}$ poise) of such a body is due. In two other dimensions however, the atom or group is "liquid", i.e. clings to its neighbours by bonds such as occur in liquids, where the internal friction is less than 10^8 poise. This state of aggregation, which occupies a definite place between the solid and the liquid states, may be conveniently referred to as the "rubber-like state."

Returning to case (*c*) (p. 31), it has been shown for a number of substances with long-range elasticity, that $\left(\frac{\partial E}{\partial l}\right)_T$ is negative; i.e. during contraction the internal energy increases. The latent heat absorbed may be compared with heat of fusion (since dissociation or evaporation are excluded). In all substances with rubber-like elasticity which are chemically well-defined, it has been found that the melting on contraction, inferred on thermodynamic grounds, received confirmation from the changes in the X-ray diffraction pattern. Thus in the case of elastic sulphur, rubber, polyphosphonitrilic chloride, etc., ordered regions giving rise to crystal interferences appear on stretching and melt during contraction. This behaviour is only shown in that range of temperature and elongation over which $\left(\frac{\partial E}{\partial l}\right)_T = a$ is negative.

The Application to Muscle

It is clear that the thermodynamical and statistical treatment of the elastic properties of an object may lead to far-reaching conclusions about its molecular structure. If now we wish to apply the same methods to the case of muscle, it must be realized at the outset that such conclusions may only be drawn in the case of a system in equilibrium with the applied force. It is therefore necessary to exclude from the measurements changes due to viscous flow, and further to avoid any stimulation which may lead to variations in the tension developed.

If these conditions are observed such conclusions may be drawn. They will concern that part of the muscle which is responsible for the development of tension on stretching and they will apply to all the components of this elastic system which are in action at a given moment. Thus if at small elongations the connective tissue fibres are slack, so that they do not contribute to the tension developed, the values obtained for the temperature coefficient of the force will concern the rest of the system (e.g. the muscle fibres) which is responsible for the tension developed under these conditions. If the connective tissue fibres are stretched at greater elongations, they may come to determine the elastic properties of the muscle as a whole. Those parts which do not contribute to the mechanical cohesion of the muscle, e.g. the tissue fluid, do not enter into consideration. In other words the thermoelastic phenomena observed represent the sum of all the changes in all the components of the elastic system, components which are not necessarily homogeneously deformed.

It is therefore impossible, without information from other sources, to associate directly the elastic phenomena and thermodynamic conclusions with definite histological elements; nevertheless, these conclusions have a certain advantage over the results of observations of birefringence or other physical properties, since they concern indubitably those elements which exert tension, and it is precisely these which are of interest to the physiologist.

In the study of the thermoelastic properties, the investigation of the temperature coefficient of the elastic force at constant elongation has been chosen as the most suitable function for our purposes. This is related thermodynamically to two other functions: the temperature coefficient of linear expansion under constant load, and the change in temperature on stretching adiabatically at constant load. An object which, like rubber at moderate elongations, shows a positive temperature coefficient of the elastic force has a negative coefficient of linear expansion at constant load, and loses heat when stretched isothermally (or is warmed by an adiabatic stretch).

A resting muscle is also such an object, and, as Heidenhain (1863) showed, is warmed on stretching. Although various workers contradicted Heidenhain's observations, the increased sensitivity of experimental methods has led to their complete confirmation.

Hill and Hartree (1929) measured the heat produced on stretching a frog's sartorius muscle, and calculated the coefficient of linear expansion.

Wöhlisch and Clamann (1931) measured the coefficient of linear expansion at constant load, and confirmed the suggestion already made on thermodynamic grounds, that this was negative for small elongations (up to 30 % of the resting length). They observed, however, that for greater initial elongations (30–40 %) the coefficient was positive.

Feng (1932) redetermined the heat evolved during small moderately slow stretches, and found that the effects were reversible under these conditions; that is to say, the muscle was warmed by stretching and cooled by shortening. With greater initial loads Feng confirmed the change in sign of the coefficient observed by Wöhlisch and Clamann, since he found that under a load of 10 g. a frog's sartorius muscle is cooled by stretching, and that this effect is also reversible. Feng suggested that the reversal in sign of the coefficient at high initial tension was due to the increased extension of the connective tissue supporting the muscle fibres—a tissue which he showed to possess a normal positive coefficient of expansion, since it was cooled by stretching.

While the properties of muscle have been studied in this way for many years (a complete survey of the literature is given by Wöhlisch 1932), the significance of these thermoelastic properties from the point of view of intimate molecular structure has been almost entirely ignored. The completion of the thermoelastic data and the discussion of their molecular significance form the content of this paper. Determinations of the temperature coefficient of the elastic force at constant length have been made, and, in addition, the changes occurring in muscle as a result of stretching have been followed by a study of the X-ray diffraction pattern.

2—Experimental Method, Material and Treatment

The immediate aim of the experiments being to determine the temperature coefficient of the force exerted by a muscle stretched at constant elongation, we have used the dynamometer of Polanyi (1925), already used for work on the thermoelastic properties of rubber and other substances. In this instrument, a change in the force exerted by a stretched object modifies the curvature of a steel spring. This change in curvature is then magnified by a system of optical levers. By this means it is possible to measure tension changes in the object under practically isometric conditions. In our experiments it was possible to measure forces up to 60 g. wt. with an accuracy of ± 0·01 g.

Because of the viability of frog's muscle, the ease with which preparations can be made, and the familiarity of the material, sartorius muscles, mostly of *Rana temporaria* but occasionally also *R. esculenta* and always from winter frogs, were used in the first experiments. A few experiments were made with gastrocnemius preparations, but the results, while of the same general character as those obtained from the sartorius, were less satisfactory, owing to (*a*) the large amount of tendon in the gastrocnemius, (*b*) the bulk of the muscle, which retards thermal equilibration, and (*c*) the non-parallel arrangement of the fibres. The sartorius, on the other hand, has (*a*) small development of tendon, (*b*) a large surface for its bulk, and (*c*) its fibres are for the most part parallel.

The muscle was dissected out with two attachments—the head and a portion of the shank of the tibia, and half the pelvis. These fragments of bone were gripped by the clamps of the Polanyi apparatus. A frog's Ringer solution containing glucose and freshly made up for each experiment was used as medium for the muscle.

The solution was heated directly by a spiral of manganin wire in a vertical glass tube perforated at the level of the liquid in the experimental vessel. A stream of air entering at the bottom of this tube produced a circulation of liquid over the heating spiral and out into the surrounding solution through the perforations; in this way also the solution was aerated. In order to avoid stimulation of the muscle by the heating current, the levelling screws of the apparatus were separated by rubber pads from their metal bed—to avoid a completion of the circuit to earth through the muscle—and a coil of copper wire was wound round the outside of the heating tube in order to short-circuit any difference of potential between the top and bottom of the solution. Before these precautions were taken, a gastrocnemius muscle had shown occasional twitches when the heating circuit was completed; after these precautions the effect was never observed. The solution could be cooled by passing iced water through a glass spiral immersed in the liquid. With this system of heating and cooling the temperature of the medium could be raised from 10 to 25° C. in 6 min., and cooled over the same range in about 15 min.

In no case was the muscle heated above 25° C. Wöhlisch and Clamann used a greater temperature range extending to 32 and even to 35° C. In our experience there is considerable likelihood of the muscle being damaged by exposure to temperatures above 25° C. for an extended period; as it was sometimes necessary to keep the muscle at the maximum temperature for several hours, the upper limit of the temperature range was fixed at 25° C. The experiment usually lasted about 12 hr., and at the end of that time the preparation was always excitable by gentle faradic stimulation.

For the experiments on the anterior byssal retractor of *Mytilus edulis*, a preparation was made with one end of the muscle attached to the shell and clamped in the apparatus. The other end of the muscle remained attached to the foot, which was tied in a loop of cotton and secured in the other clamp by this means. Sea water was used as medium.

3—THERMOELASTIC PHENOMENA

(*a*) *Experiments on Frog's Muscle*

It has already been pointed out that the purely elastic phenomena can only be investigated in a system in a state of equilibrium, since we wish to observe changes which are reversible in the thermodynamic sense. Experiments made on the viscous-elastic and thermoplastic phenomena exhibited by muscle (to be described later) have indicated the precautions which must be taken in order to approach equilibrium conditions. They show that it is best to allow the muscle to reach a steady tension at the highest temperature and the greatest load before proceeding to the examination of the thermoelastic properties.

The behaviour of frog's sartorius muscle at medium elongation is shown in fig. 1. This is a good example of the results obtained by changing the temperature of a muscle in which the force had reached a steady value. The figure shows simultaneously the change in temperature and the change in elastic force with time at an initial force of 1·51 g. It is apparent that the temperature coefficient of the force exerted is positive, as was predicted on thermodynamic grounds. Fig. 2 shows the effect of two temperature cycles at a higher initial force of 2·40 g. In this case the fall in tension is still incomplete, as can be seen from the changing base-line, and at the higher temperature there is a tendency for the tension to fluctuate (probably as a result of convection disturbance). This effect was frequently observed, during experiments with non-living material as well as with muscle.

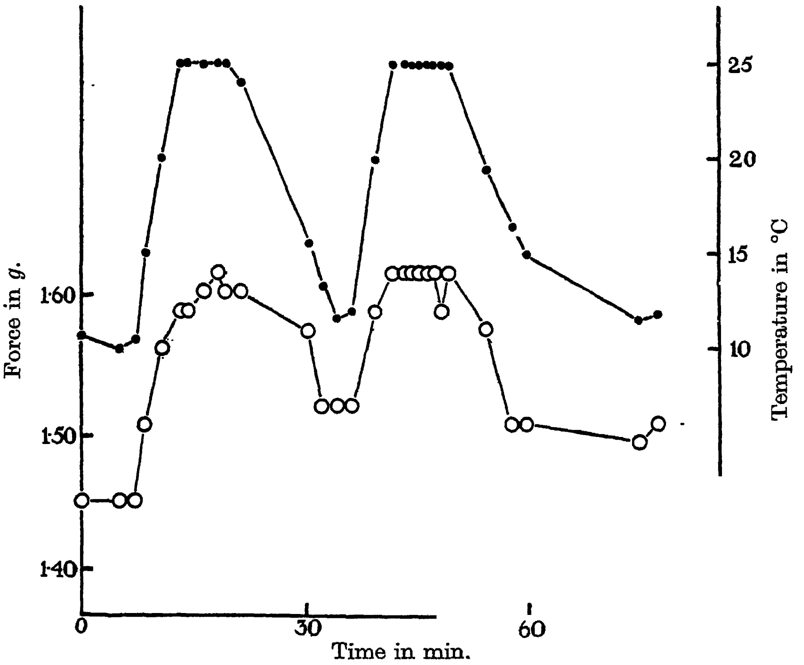

Fig. 1—The effect of a change in temperature on the force exerted by a frog's sartorius muscle under a load of 1·51 g. and at an elongation of *ca.* 15 %. ○ = force, ● = temperature.

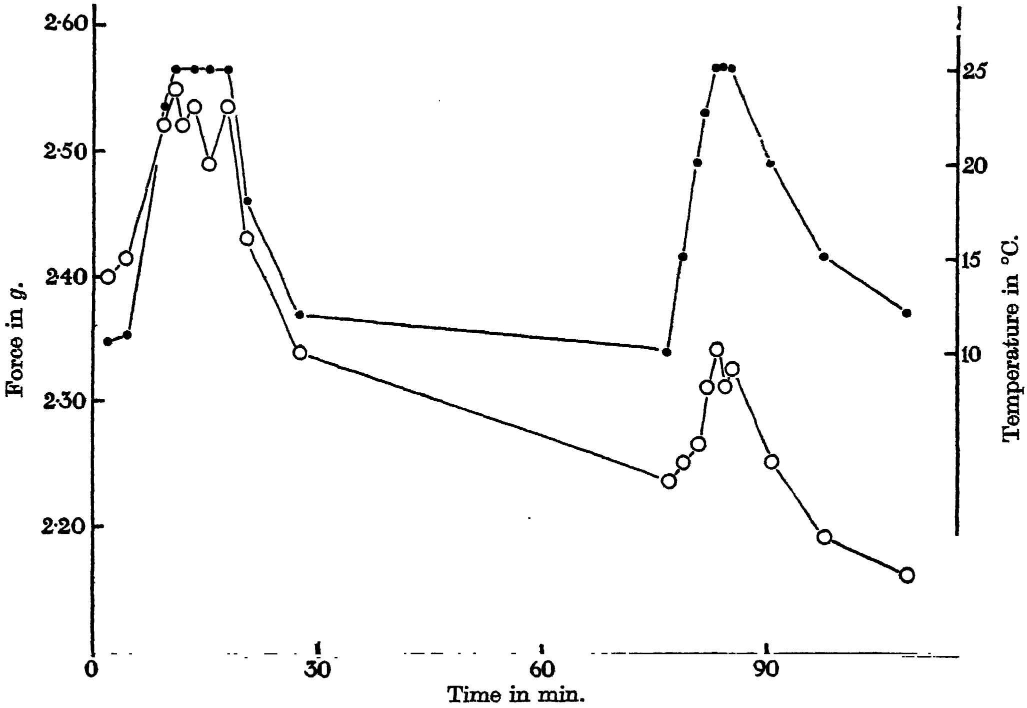

Fig. 2—The effect of a change in temperature on the force exerted by a frog's sartorius muscle under a load of 2·40 g. and at an elongation of *ca.* 40 %. ○ = force, ● = temperature.

At forces less than 1·5 *g.* the temperature coefficient becomes first zero (see latter part of fig. 6, force = 0·38 g.), and at still lower initial loads (0·35 g.) the coefficient is negative, as in fig. 3. Fig. 4 summarizes the results for one muscle at five different elongations; it is clear that this figure is entirely similar to that which may be obtained from rubber (see Meyer and Ferri 1935, p. 578).

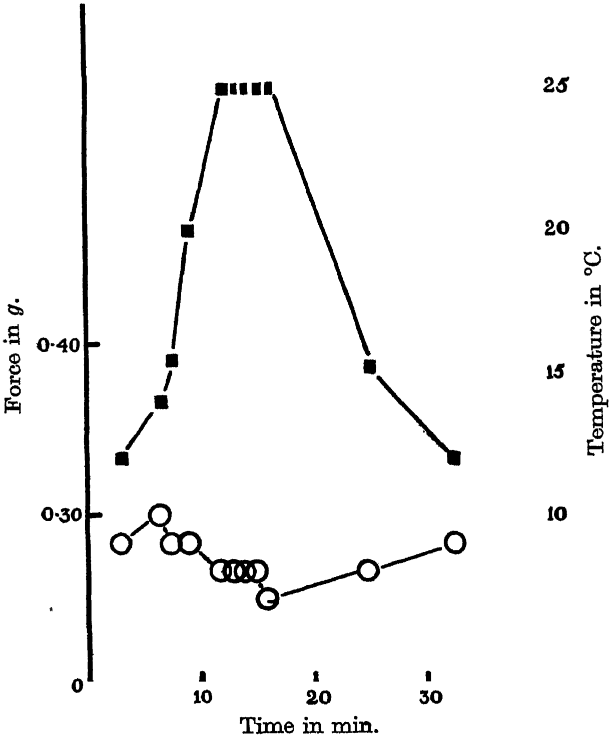

Fig. 3—The effect of a change in temperature on the force exerted by a frog's sartorius muscle under a load of 0·30 g. and at an elongation of *ca.* 10 %. ○ = force, ■ = temperature.

The observations of Wöhlisch and Clamann, and also of Feng, that a muscle which has been allowed to pass into rigor has a positive coefficient of linear expansion, was confirmed by the negative coefficient of the elastic force obtained for such a muscle in the course of these experiments.

Wöhlisch and Clamann attributed the positive coefficient of linear expansion, which they found at very small elongations, to the changes in density of muscle and medium. In the Polanyi apparatus such an error was found to be inconsiderable; the change in density of the solution between 10 and 25° C. was insufficient to produce a detectable change in the apparent weight of the clamp, as measured by the displacement of the scale. Only a change in tension therefore could produce the observed movements of the scale. Accordingly the change from a positive to a

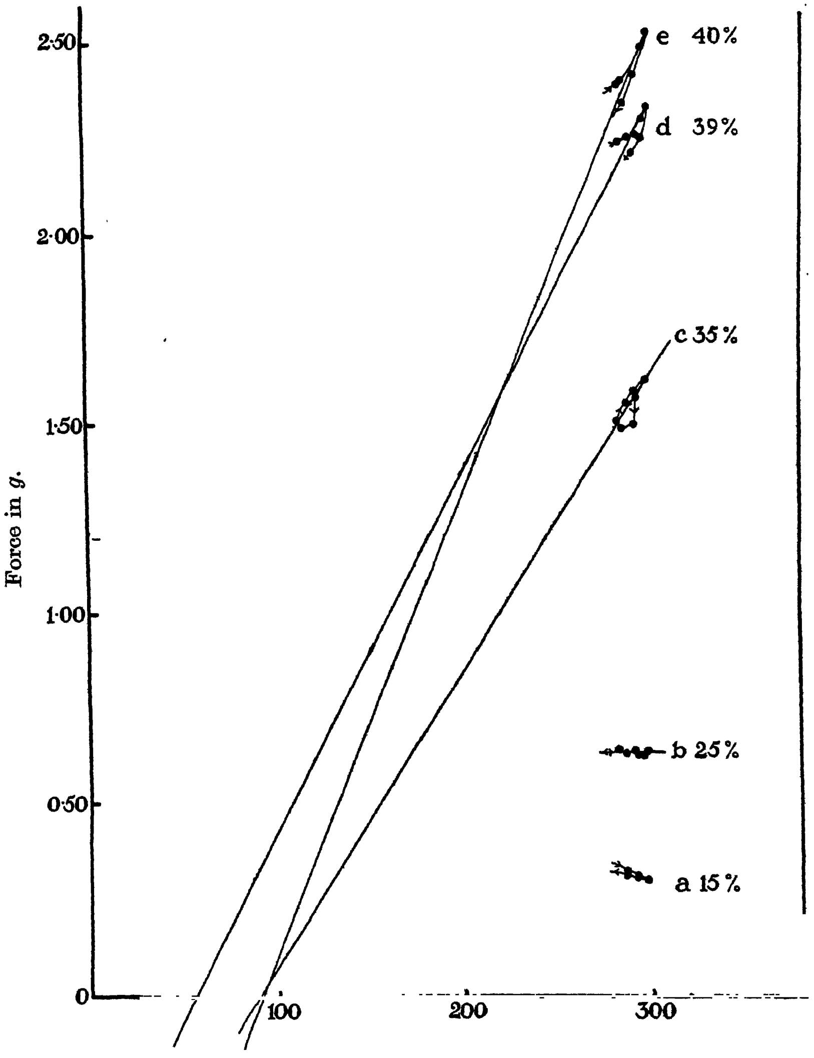

FIG. 4—Curves showing the elastic force, exerted by a frog's sartorius muscle at five different elongations, plotted as a function of the absolute temperature. Since in curves *d* and *e* the initial and final values for the elastic force do not coincide, the tangents are drawn to the upper portion of the ascending limb.

zero coefficient is real, and the observations are not due to properties of the apparatus.

Fig. 5 *shows the behaviour of the muscle when the elastic force is zero.* (Zero-tension-length was determined by plotting force as a function of elongation, and so finding the length at which the tension first began to rise.) It is apparent that at zero tension the coefficient is at least not positive. Other experiments with the muscle under a very small load showed a fall in the force exerted as the temperature increased, i.e. a negative coefficient at a load of *ca.* 0·01 g.

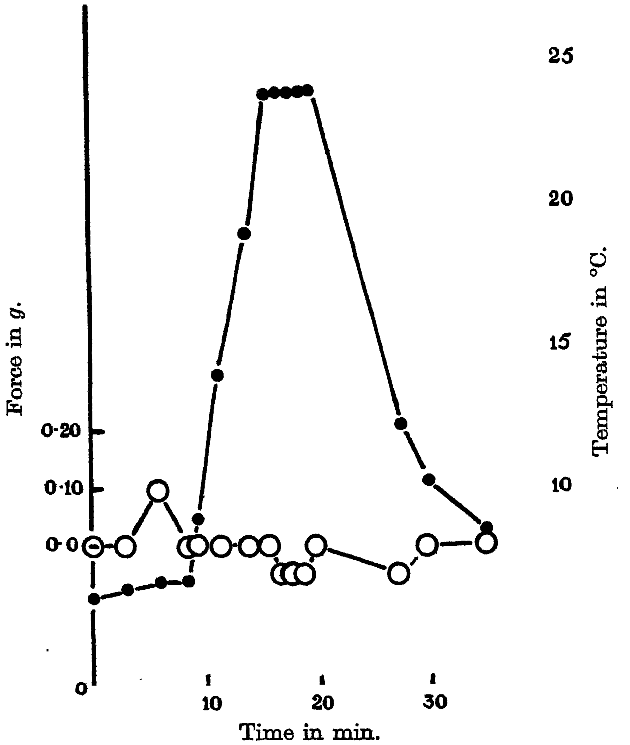

Fig. 5—The effect of a change in temperature on the force exerted by a frog's sartorius muscle under zero load and at resting length (= 0 % elongation). ○ = force, ● = temperature.

These experiments therefore indicated a negative coefficient of the elastic force at zero tension, exactly as in the case of rubber and other high polymers, and differing, for example, from that of elastic tissue (Wöhlisch 1926) which has a positive coefficient at zero and medium elongations.

It was sometimes observed, in attempting to repeat force-temperature cycles at very small tensions, that the coefficient had changed in sign, and the change was always from a negative to a zero or a positive coefficient, whether the low tension was approached from below or from above. This phenomenon is shown in fig. 6 from a muscle which had rested for several hours in cool, stirred Ringer solution at zero tension.

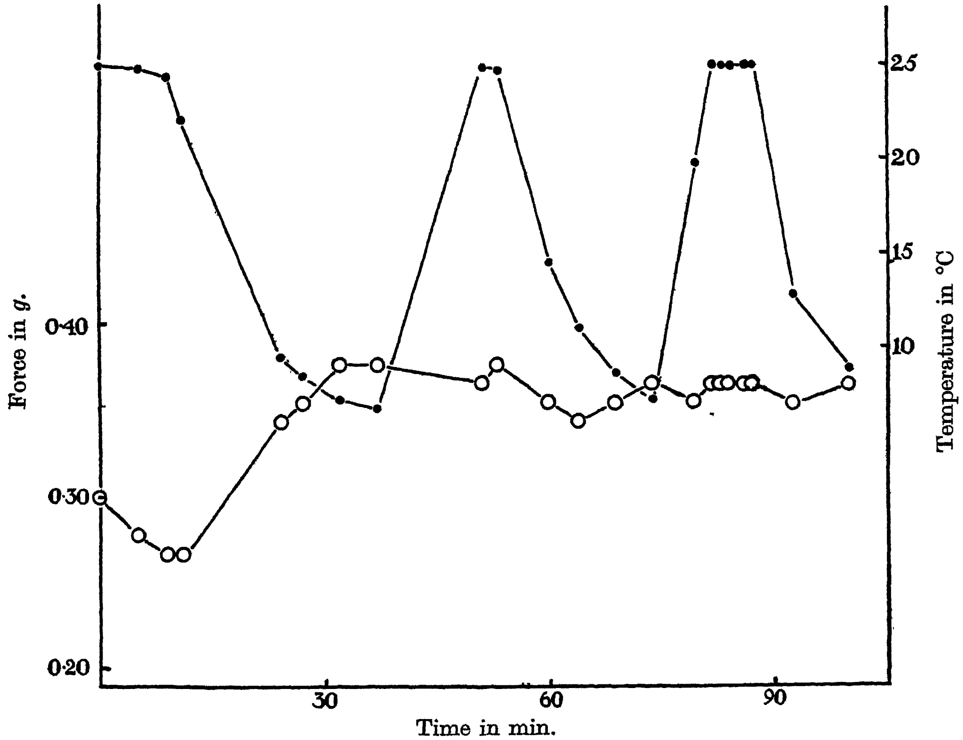

FIG. 6—The effect of a change in temperature on the force exerted by a frog's sartorius muscle under a load of 0·30 g. and at an elongation of *ca.* 10 %. This figure shows the spontaneous change of the temperature coefficient of the elastic force from negative to zero values. ○ = force, ● = temperature.

The behaviour of muscle at greater elongations is shown in fig. 7 obtained from a muscle stretched for 24 hr. at a load of about 12 g. The coefficient is negative, and rather smaller than the positive coefficient (as Feng and Wöhlisch found). This muscle was still excitable.

The reversibility of the change in sign of the coefficient from positive to negative is illustrated in fig. 8. After a cycle of temperature change at an initial force of 1·66 g., the muscle was stretched until the force rose to 10 g.

at which load the coefficient is negative; this load was maintained for 5 min. On returning to the original tension, the second cycle of temperature change was completed, and differs chiefly from the first in the change in base-line due to the rise in tension which follows a sudden shortening.

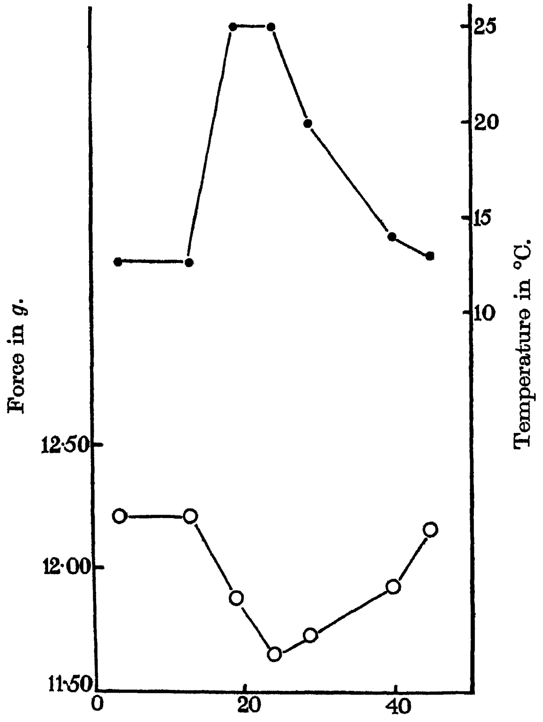

Fig. 7—The effect of a change in temperature on the force exerted by a frog's sartorius muscle under a load of *ca.* 12 g. ○ = force, ● = temperature. Elongation *ca.* 45 %.

It is therefore clear that the change from a negative to a positive temperature coefficient of the elastic force at small elongations, and the reverse of this phenomenon at greater elongations, are both real and reversible processes.

(*b*) *Experiments on Muscles from* Mytilus edulis

These experiments were much less satisfactory than those made with frog's sartorius muscle because of the absence of two rigid attachments to the muscle, and also because of its greater plasticity. They showed, however, that the temperature coefficient of the force exerted was positive at moderate elongations, just as in the case of frog's sartorius.

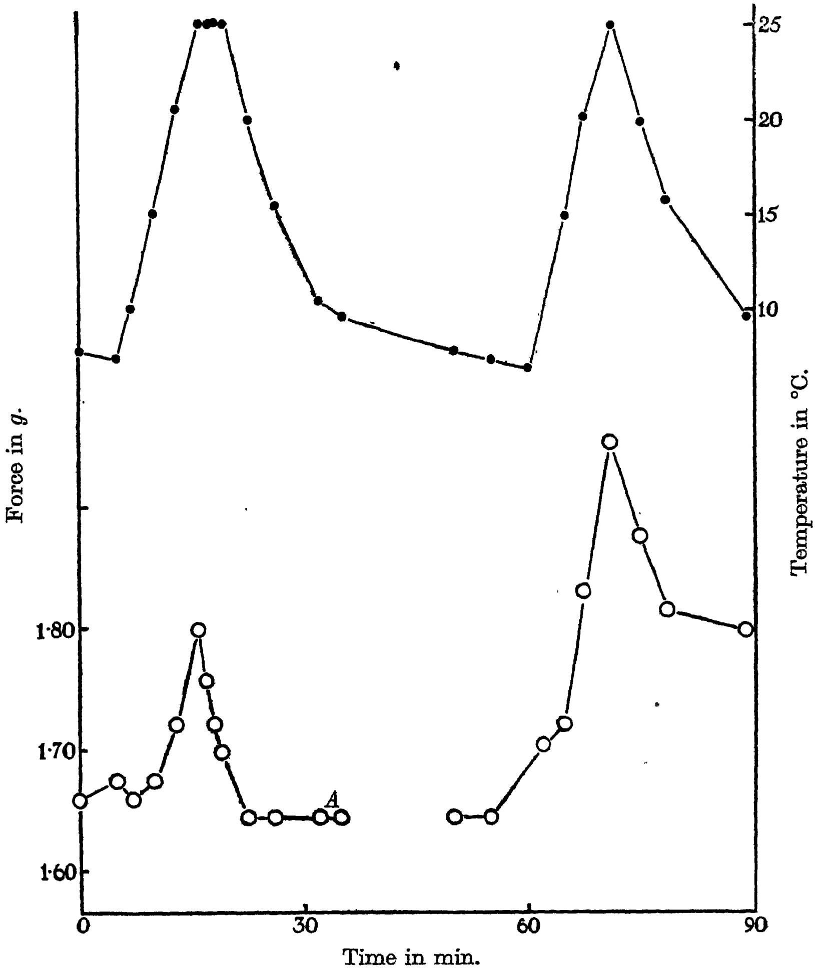

Fig. 8—The effect of a change in temperature on the force exerted by a frog's sartorius muscle at moderate elongation (*ca.* 35 %), before and after a large stretch. At *A* the load was increased to *ca.* 12 g. and maintained at this value for 5 min. ○ = force, ● = temperature.

4—THE MOLECULAR SIGNIFICANCE OF THE THERMOELASTIC PROPERTIES OF MUSCLE

It is evident from these results that the mechanism of elastic contraction of a stretched, unstimulated muscle is entirely similar to that of all other substances with rubber-like elasticity whose thermoelastic properties have been examined. In particular, it is apparent that the discrepancy between the thermoelastic behaviour of muscle and that of rubber, postulated by other workers (i.e. that the temperature coefficient of linear dilatation in rubber is positive at small elongations, and negative at higher elongations, whereas in muscle the converse is true), is illusory.

Both muscle and other substances of rubber-like character at small elongations have the thermoelastic properties of a normal elastic solid, i.e. the temperature coefficient of the elastic force is negative, and the object behaves as a glass or as a crystal, in which deformation takes place with the displacement of atoms or groups from positions of minimal potential energy, and contraction results from the increase in potential energy which occurs when the system is deformed.

At intermediate elongations, however, in both muscle and rubber-like polymers, the temperature coefficient of the elastic force is positive, and therefore augmentation in potential energy on stretching cannot alone account for contraction. Since the coefficient is positive, the constant b in equation (3) p. 30 is also positive, and as $b = -\left(\frac{\partial S}{\partial l}\right)_T$, it is clear that isothermal stretching of the muscle produces a diminution in the entropy of the system, i.e. the arrangement of the atoms or groups becomes more ordered and less probable. A glance at fig. 4 shows, further, that in the range of elongation over which the temperature coefficient is positive, the tangent to the curve representing force as a function of the absolute temperature makes a negative intercept on the K axis (curves c, d and e), i.e. a in equation (3) is negative, and therefore the internal energy, far from increasing during stretching, diminishes rather. This means that during an isothermal stretch the increase in orientation proceeds with evolution of latent heat; in other words, a sort of crystallization takes place as a result of stretching (see case (c) p. 31).

It has been pointed out already (p. 32) that the mere fact of an object possessing elasticity of the rubber type enables us to say that the object is composed (in part at least) of long, flexible chain molecules, built up of links which are firmly bound to each other. By rotation about any single homopolar bond of the chain, the distance between links of the same chain may be varied, while the energy content remains unchanged. Thus an irregularly twisted chain can be straightened out without any change in internal energy, and with change in entropy only.

We conclude therefore that the elastic system of the muscle also consists in part of flexible chain molecules.

Let us now consider the behaviour of this system at different elongations.

In the resting condition the chains are twisted into configurations of maximum probability, and the effect of a small stretch is to separate the atoms or groups very slightly (work may be done against van der Waals forces). At this elongation the temperature coefficient of the elastic force is negative (as in the case of an amorphous substance such as a glass).

As deformation continues, the straightening of the twisted chains becomes more and more significant, and the temperature coefficient of the force exerted becomes first zero and later positive, since the effect of thermal agitation on the form of the chains becomes increasingly important as a component of the elastic force. In the case of muscle we have seen that the orientation of the chains proceeds until an evolution of heat occurs and a sort of crystallization takes place. On allowing the muscle to contract the "crystallites" melt.

Finally, at high elongation, the chains (or at least a part of them) are fully stretched and completely orientated, and further elongation by rotation about the primary bonds is impossible, i.e. elongation can only take place if the kinetic units are displaced from their troughs of minimal potential energy. Stretching is therefore accompanied by an increase in the potential energy of the system, and the sign of the temperature coefficient of the elastic force becomes negative once more. The system behaves like a crystalline aggregate in which orientation is complete, and stretching can only lead to an increase in potential energy.

The change in sign of the temperature coefficient observed in muscle at high elongation has probably still another cause. Feng has pointed out that the connective tissue fibres begin to participate in the tension at greater deformations, and since the temperature coefficient of linear expansion of these is positive, it is clear that at high elongations the positive coefficient of the force due to the muscle substance will be masked by the increasing importance of the negative coefficient due to the connective tissue. We can say, in any case, that at high elongation the behaviour of the muscle is that of a system in which chain molecules are maximally stretched and orientated. It is not possible to say as yet how far the different elastic properties are due to the fibre substance, to the connective tissue fibres, to the sarcolemma, or to any other histological component.

5—THE X-RAY DIFFRACTION PATTERNS OF LIVING MUSCLE

The study of the thermoelastic properties of muscle has indicated that the increased orientation of chain molecules, which takes place during isothermal stretching of the muscle, proceeds with the evolution of latent heat. Since it has been possible in the case of certain rubber-like high polymers to confirm the occurrence of such "crystallization" as a result of deformation, by examining the X-ray diagram of the substance in question, a study of the diffraction pattern of muscle was undertaken from this point of view.

X-ray photographs were taken with a Siemens fine-structure tube with Cu anode, operating at 30 kV and 17 mA. The radiation was filtered by a sheet of 0·02 mm. Ni foil, so that the interferences were due chiefly to the $K\alpha$ band of the Cu radiation. Photographs were taken using a pin-hole collimator, 1 mm. in diameter and 6·0 cm. long, the distance from muscle to film being generally between 2·5 and 3·0 cm. Under these conditions an exposure time of 1½ hr. was necessary.

The muscle was kept wet by a stream of Ringer solution (in the case of frog's muscle) or sea water (in the case of mussel and rock-lobster muscles). In the first experiments the muscle was moved continuously through the X-ray beam (following the recommendation of Boehm 1931), so that the destructive effect due to irradiation might be reduced as much as possible, and a fresh region of the muscle presented continuously to the beam. Experiments showed, however, that it was not possible to distinguish between the diagram obtained in this way and that obtained from a stationary muscle. Since it was necessary to control both the temperature and the elongation of the muscle, it was decided (for the sake of simplifying the mechanical arrangements necessary) to take all photographs with the muscle stationary. In order to control the temperature of the muscle, a brass box which could be filled with ice or warm water as required was built round the cap of the pin-hole collimator. The muscle was laid over the pin-hole—the penetration of solution into the latter being prevented by a thin film of nitrocellulose, attached to the box by shellac varnish. This film also served to prevent direct contact between the muscle and the metallic surface.

One end of the muscle was fixed and the other attached to a thread passing over a pulley; the elongation could then be increased by adding weights.

It was found that the surface of the muscle easily became dry, although a stream of Ringer solution was directed on to one end of the muscle. To prevent local desiccation during an exposure, the surface of the muscle was covered by a thin film of nitrocellulose.

Under these conditions it was possible to keep the muscle at a temperature of *ca.* 3° C. for 1½ hr. if the metal box were filled with ice, and the solution irrigating the muscle had been previously cooled on ice to about 8° C. The temperature could be raised and kept at 25° C. by replacing the ice with water and heating the box with a micro-burner, while the Ringer solution could be kept at the necessary temperature by an electric lamp dipping into the liquid in the reservoir. The muscle was always earthed through the metal box.

(*a*) *The X-ray Diagram of Muscles from Animals belonging to different Phyla*

Owing to the presence of convenient attachments, the sartorius muscle of the frog was used for experiments on the effect of stretching and temperature change on the X-ray diagram; but in order to determine how far the diffraction patterns of different kinds of muscles are comparable, and to what extent the essential molecular structure is independent of histological differences, comparative studies of muscles from *Mytilus edulis* and from *Palinurus elephas* were made as well.

The results of these showed that the diagram is essentially the same for frog's sartorius, for the anterior byssal retractor and posterior adductor muscles of the mussel, and for muscles from the thoracic limbs of the rock-lobster.

The X-ray diagram of the living muscle (fig. 9, Plate 3), shows, as Boehm (1931) described (for earlier references see Boehm), a ring (corresponding to a spacing of about 10 A) with an accentuation in intensity forming a diffuse spot on the equator. From our photographs it can be seen that the spot is drawn out equatorially into a continuous streak, passing into the primary beam in a manner suggesting that other interferences of smaller glancing angle are present, corresponding to spacings much larger than 10 A. This streak cannot be due to the white radiation, since it is absent from the diagram of dried muscle. From this fact it follows that the presence of water is essential for this appearance.

External to the ring are a pair of meridian arcs, not mentioned by Boehm, although traces of them are visible in the reproductions of his photographs. These correspond to an identity period of *ca.* 5 A in the direction of the fibre axis. The behaviour of the inner ring and the meridian arcs in the experiments which have been made leads one to suppose that both originate from the same substance.

The meridian sickle is enclosed by a second ring, largely due to water.

Of the three types of muscle examined, the crustacean muscle gave the poorest fibre diagram. In this case the equator point was just visible, and the meridian arcs were drawn out into a circle. *Mytilus* muscles, on the other hand, gave the best diagrams; the equatorial streak and the meridian arc were always very well developed. Any differences between these diagrams lay within the limits of error of measurement, i.e. within ±10 % of the values for the spacings given here.

The X-ray photographs of all these muscles after drying under moderate tension, washing in distilled water and redrying, could be distinguished from each other by the development of the meridian arc, which was most marked in *Mytilus* muscle (fig. 11, Plate 3), less in frog's sartorius, and scarcely to be seen in *Palinurus*. The positions of the interferences did not differ significantly from their positions in the photographs of fresh muscle. It was particularly noticeable that the indications of interferences lying near the primary beam disappeared on drying. Photographs of dried *Mytilus* muscle, taken at a plate-object distance of 7·6 cm., showed no trace of interferences between the equatorial spots corresponding to a spacing of *ca.* 10 A and the central spot. In the case of dried muscles, the estimation of spacings was accurate to ± 5 %. Differences between the diagrams from muscles of different animals lie again within the limits of error.

The uniformity of these results suggests that in each case a very similar molecular structure is responsible for the X-ray diagram. In view of the similarity between the diagram given by myosin fibres and that of muscle itself (Boehm and Weber 1932) we may reasonably suppose that it is the muscle fibrils themselves which are responsible for the diffraction pattern, since they are the only histological component common to all three types of muscle.

(*b*) *The Effect of Stretching on the X-ray Diagram of Frog's Sartorius Muscle*

As a preliminary study the effect on the diagram of the thickness of muscle exposed to the beam was investigated. A sartorius was placed before the pin-hole in one experiment so that the thickest region of the muscle (near the insertion on the head of the tibia) was irradiated; in another experiment, the edge of the same muscle (in the region of the pelvis, i.e. the thinnest portion of the muscle) was laid over the pin-hole. The diagrams so obtained were indistinguishable. It was therefore concluded that the change in thickness of the muscle as a result of stretching would not alone produce any change in the X-ray diagram, i.e. the effect observed must be due to changes in orientation of the muscle substance.

The effect of varying the amount of solution passing over the muscle was also examined, and no difference in the intensity of the water-ring could be observed, whether the muscle were continuously irrigated or moistened at intervals of 10 min. It appeared therefore that small variations in the flow of the physiological solution would not influence the X-ray diagram.

These experiments showed that the equatorial point becomes somewhat sharper, and darker relative to the ring, when the muscle is stretched from 0 to about 35 % (the temperature being constant at about 15° C.). Similarly the meridian arc was better defined in the stretched than in the unstretched muscle. The effect, however, was very slight.

(*c*) *The Effect of temperature Change on the X-ray Diffraction Pattern of Frog's Sartorius Muscle*

If stretching results in an increased orientation of the muscle substance with liberation of latent heat, it should be possible, by raising the temperature, to diminish the orientated component of the system, and therefore to produce a diminution in the sharpness and intensity of the interferences of the X-ray diagram.

In these experiments the muscle was cooled slowly under moderate tension (*ca.* 15 g. load) to *ca.* 3° C., cooling taking about half an hour, in order to allow a

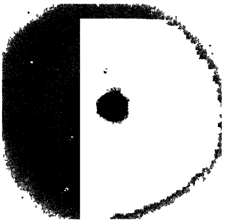

FIG. 9

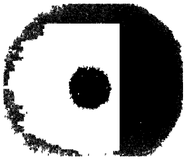

FIG. 10

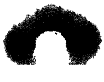

FIG. 11

maximum formation of crystallites. A photograph was taken at this temperature, and the muscle was then moved so that a fresh region covered the pin-hole, the temperature was raised to 25° C., and a second photograph was taken. This process was repeated at 30° C.

The diagram obtained at 25° C. showed a very slight diminution in definition of the equatorial point and the meridianal arc as compared with the diagram at 3° C. In the case of the photograph at 30° C. however, the equatorial point and the meridian arc had almost disappeared.

(*d*) *Conclusions to be drawn from Experiments on the effect of Deformation and Temperature Change on the X-ray Diagram of Muscle*

The results of these experiments show that in vertebrate, molluscan and crustacean muscle there is an orientated component. As compared with high polymers such as rubber, polyphosphonitrilic chloride, elastic sulphur, etc., the orientated component of muscle shows a very poor fibre diagram. The muscle diagram may, however, be compared with that of polyvinyl acetate (Misch and Picken 1937), which shows orientation without crystallization (confirmed by the thermoelastic behaviour). In the case of muscle, the diagram exhibits signs of a higher orientation of the molecules than is present in polyvinyl acetate; this suggestion is supported by the thermoelastic properties, which indicate that latent heat is set free when the muscle is stretched. In this very limited sense therefore, we can say that the muscle fibrils contain elements possessing a rudimentary crystal-like structure.

From the thermoelastic behaviour of muscle it was predicted that the amount of the "crystalline" component increases during stretching. Actually it has been shown that the slight changes in the X-ray diagram which result when the muscle is stretched are in agreement with the view that the amount of orientated substance increases in the process of stretching.

Similarly the attempt to influence the diagram by raising the temperature and so melting the crystallites has shown in fact that the fibre diagram of muscle is less pronounced at a temperature of 30 than at 3° C. (the elongation being maintained constant).

We can say therefore that the X-ray examination of the structure of muscle has supplied evidence supporting the hypothesis of a partial "crystallization" of the muscle substance, already postulated on thermodynamic grounds.

6—IRREVERSIBLE PHENOMENA

The study of the thermoelastic properties and X-ray diffraction patterns of muscle has led us to a certain conception of its molecular structure. We have seen that the elastic system appears to consist of long, flexible chain molecules, which can be orientated by stretching, and of which a certain fraction are associated to form crystallites. The same type of elastic behaviour is shown by high polymers, such as rubber, polyphosphonitrilic chloride, etc. In the case of these substances it has been possible to explain in terms of molecular structure, not only the thermoelastic properties and the changes in the X-ray diagram, but also irreversible phenomena of deformation, such as relaxation, the thermoplastic effect and hysteresis; it is the object of this chapter to consider such phenomena as they are shown by muscle, and to indicate their molecular significance.

(a) *Viscous-elastic Phenomena*

These effects were first studied by Gasser and Hill (1924) on the tetanized sartorius muscle of the frog. They have since been investigated in the resting sartorius by Pochin (1935), who followed the change in length with time of loaded and subsequently unloaded muscles. Similar experiments have been made by us, but following the change in elastic force with time. (The difference between the effects observed with the tetanized sartorius and those shown by the resting muscle, lies chiefly in the time scale.) Entirely similar effects can be observed in the case of certain high polymers, e.g. slightly vulcanized rubber, and the description of viscous-elastic properties given here may be applied both to these and to muscle.

When the object is stretched rapidly and kept at constant length, the tension falls, but eventually becomes constant. If the length is now suddenly reduced, the tension at first diminishes rapidly, but later rises in time, until a maximum value is reached, after which it begins to fall once more, but now very slowly.

These effects might be imitated by a system such as the viscous-elastic model proposed by Gasser and Hill. But this model does not offer a molecular explanation of the phenomenon involved.

In order to explain this behaviour in terms of molecular structure, we must first consider briefly the phenomenon of relaxation, as exhibited by well-known and simpler chain polymers. *The term relaxation is used to denote the fall in tension in time which is observed when a given object is stretched and the length kept constant. Since we are considering only the resting muscle, the term is employed here in an entirely non-physiological* *sense*; the muscle is treated simply as a viscous-elastic system, like a piece of rubber or polyvinyl acetate.

All elastic substances, consisting of long chain molecules which are not united by cross-linkages to form a network, show complete relaxation when held stretched at constant length for a sufficiently long period, i.e. the tension falls to zero. As the elastic force diminishes, the double refraction and (in the case of those substances showing partial crystallization) the sharpness and intensity of the interferences of the X-ray diagram are also reduced. This means that the chains which were at first orientated by stretching, become disorientated: from being straightened out they contract and become irregularly twisted, sliding over each other until their configurations are chaotic and highly probable, as in an amorphous, unstretched object.

If, on the other hand, the chains are united by chemical bonds to form a loose, three-dimensional network, this unrestricted sliding over each other becomes impossible. Such is the case in considerably vulcanized rubber and elastic fibres. Many high polymers, however, show a mixed type of structure: two interpenetrating systems are present, a network which cannot relax, and chains which show relaxation. This is the condition in slightly vulcanized rubber, polyphosphonitrilic chloride, polyvinyl acetate, etc. We have seen that the muscle resembles these substances in that both show limited relaxation. It is concluded therefore that in muscle, also, the viscous-elastic properties are due to the presence of a network of chains, in the meshes of which are free chains. This conclusion is supported by the results of preliminary experiments on the birefringence of frog's sartorius muscles and buccal retractor muscles of *Helix pomatia*. If the muscle is stretched rapidly and held at constant length the double refraction of the stretched muscle diminishes in time, as in the case of certain high polymers such as polyvinyl acetate.

When such a system is stretched, the chains are straightened out and orientated: those of the linked-chain system since they behave like a three-dimensional net subjected to a non-homogeneous tension, the others because of the frictional drag exerted by the first system. But while the network is held fast the free chains are able to slide until their distribution is once more chaotic; correspondingly, the tension and the double refraction fall in time until this distribution is complete.

As soon as the tension is reduced the network contracts and the free chains are now strained in the direction of contraction. There will thus be at first an apparent equilibrium between the tendency of the network to contract, and that of the free chains to expand. But since the latter are able to move freely they will gradually yield to the constraint exerted by the network, and further contraction of the latter leads to a rise in tension of the object.

The hypothesis of the existence of two systems in slightly vulcanized rubber (advanced by various investigators) is supported by the fact that part of the rubber is soluble in organic solvents (the free chains), leaving an insoluble residue.

Similarly, in the case of muscle it has been shown by Saxl (1907) that it is possible to remove nearly 90 % of the total protein in soluble form by the repeated extraction of fresh and finely minced muscle.

It is reasonable to suppose, then, that the behaviour of muscle is determined by the existence of two systems, one of which has elastic and the other plasto-elastic properties. This conclusion is not very different from that drawn by Gasser and Hill from their study of the active muscle. We believe, however, that it is possible to identify these two systems with the three-dimensional network of primary-valence chains, and the free chains in the meshes of this network. In the present state of our knowledge it is impossible to associate these systems with any particular histological elements in muscle.

(*b*) *Thermoplastic Phenomena*

At high temperature the rate of fall in tension of a stretched muscle at constant length is greater than at low temperature, and it was necessary to take this fact into account in the experiments on the thermoelastic properties. In some cases, when the muscle had apparently reached a steady tension at a given elongation, it was observed that the tension began to fall when the temperature was raised. This is shown in fig. 8 by the first cycle of temperature change, in which the elastic force diminished rapidly at the highest temperature before the muscle was cooled. In the case of this muscle the steady tension had been reached at the lowest temperature. It was therefore always desirable to allow the muscle to reach equilibrium at the highest temperature to which it would be exposed during the experiments on its thermoelastic properties.

The same effect is shown by certain high polymers, e.g. slightly vulcanized rubber, and the phenomenon may be explained in terms of the model already suggested. At high temperature, the plasticity of the free-chain system is greater than at low temperature; in consequence relaxation comes sooner to an end, and equilibrium is established more rapidly.

(c) *Hysteresis Phenomena*

The force-deformation curve of muscle was also examined. It was found that if the maximum load did not exceed 3 g., the muscle showed no measurable permanent deformation, i.e. the initial and final states coincided, although the curve of extension did not coincide with that of contraction. This work-loss in a cycle of loading-unloading, i.e. the hysteresis, is shown by many high polymers, and indicates that the deformation has not proceeded sufficiently slowly for the establishment of true equilibrium. Experiments at different temperatures showed that the area of the hysteresis loop was less at a temperature of 20° C. than at 9° C. (as is the case with high polymers such as rubber and polyphosphonitrilic chloride).

The hysteresis may be considered as due in part to retardation in the establishment of the equilibrium position of the free chains (as a result of their high viscosity), and in part to delay in the process of "crystallization".

7—The Condition of the Chain Molecules in Contracted Muscle

We have seen that primary-valence chain molecules are present in muscle (more specifically in the muscle fibrils). The links of these chains are able to move over those of neighbouring chains like the molecules in a liquid. We may now consider whether the condition of the chains in the contracted muscle is the same as that in resting muscle.

It has already been shown by Meyer (1929) and Boehm (1931), that the orientated fraction of the muscle substance disappears during isotonic contraction. This has been confirmed by X-ray photographs of a small bundle of fibres from the posterior adductor and anterior byssal retractor of *Mytilus edulis* in the contracted condition (fig. 10, Plate 3). Microscopic examination showed that the fibres themselves were not significantly crumpled in the contracted state, i.e. the change of the fibre diagram into a Debye-Scherrer diagram is not therefore due to a microscopic disorientation of the fibres, but must be due to a submicroscopic change in the structure of the system. It is reasonable to suppose that the chains themselves are crumpled in the contracted state. Indeed, it is difficult to imagine contraction taking place without a crumpling of the chains.

The suggestion has been made (Meyer, 1929) that contraction occurs as a result of a chemical reaction causing the chains to contract. Without further discussion of this theory we wish to consider what conclusions about the condition of the crumpled chains may be drawn from thermoelastic and other properties of the contracting muscle.

Our experiments have shown that if a muscle in tetanus is rapidly cooled, the force exerted increases, i.e. the force developed has a negative temperature coefficient. Since, however, the system is not in equilibrium, it would be impossible, without other evidence, to draw any conclusion about the elastic properties of the contracting muscle from this fact.

Azuma (1924) has shown, however, that during the onset of contraction at least, the muscle is cooled by stretching, i.e. it behaves, not as a rubber-like substance, but as a normal solid body, in which deformation takes place with the displacement of atoms or groups from their positions of minimal potential energy.

This observation is in agreement with the negative temperature coefficient of the force developed already described. Both results suggest that the excited muscle, at least at the beginning of the contraction, resembles a normal solid. This means that there is a fundamental difference between this condition and the non-stimulated condition: as a result of stimulation the mobility of the links of neighbouring chains relative to each other has disappeared; the "liquid" bonds have become "solid" bonds, and the viscosity of the system has very considerably increased.

This change in the nature of the bonds present in the system is also shown by the experiments of Gasser and Hill (1924) on the damping of the vibrations of a steel spring by a muscle. The viscosity of the muscle (as measured by the damping) is much greater in the tetanized muscle than in the resting muscle.

We suggest therefore that during contraction the protein chains pass from the rubber-like state to that of a solid body. It may be said that the muscle protein undergoes a reversible coagulation.

We are deeply indebted to Dr Lore Misch for constant and invaluable assistance and advice during the work on the X-ray analysis of muscle structure.

SUMMARY

The effect of temperature change on the elastic force exerted by a stretched, unstimulated muscle at constant length has been investigated.

At small and large elongations, the temperature coefficient is negative: the muscle behaves like a normal solid.

At intermediate elongations the coefficient is positive and the elastic

force increases more rapidly than the absolute temperature. From this behaviour the following conclusions about the structure of muscle and the origin of the elastic force may be drawn:

(*a*) The elastic system of muscle (and in particular that of the fibrils) consists of flexible, primary-valence chain molecules, the arrangement of which in the resting muscle is random and highly probable, but becomes ordered and less probable in the stretched muscle. Thermal agitation tends to provoke a return to the original, unordered condition.

(*b*) Latent heat is set free during stretching and may be considered as a sort of heat of crystallization.

X-ray studies show that the degree of orientation of the muscle substance is augmented by stretching and diminished by warming—in agreement with the conception of molecular structure reached from a consideration of the thermoelastic behaviour.

A molecular interpretation of the viscous-elastic properties of muscle is offered, and is supported by experimental evidence. The elastic system of the muscle behaves as if composed of two components: flexible protein chains forming a three-dimensional network, and free chains in the meshes of this net.

While in the resting muscle the links of neighbouring chains are able to slide freely over each other, like the molecules in a liquid, in the actively contracting muscle such "liquid" bonds appear to be replaced by more "solid" linkages.

REFERENCES

Azuma, R. 1924 *Proc. Roy. Soc.* B, **96**, 338.
Boehm, G. 1931 *Z. Biol.* **91**, 203.
Boehm, G. and Weber, H. H. 1932 *Kolloidzschr.* **61**, 269.
Feng, T. P. 1932 *J. Physiol.* **74**, 455.
Gasser, H. S. and Hill, A. V. 1924 *Proc. Roy. Soc.* B, **96**, 398.
Heidenhain, R. 1863 *Zbl. med. Wiss.* **1**, 545.
Hill, A. V. and Hartree, W. 1920 *Philos. Trans.* B, **210**, 153.
Meyer, K. H. 1929 *Biochem. Z.* **214**, 253.
Meyer, K. H. and Ferri, C. 1935 *Helv. Chim. Acta*, **18**, 570.
Meyer, K. H. and Ferri, C. 1936 *Pflüg. Arch. ges. Physiol.* **238**, 78.
Meyer, K. H., Lotmar, W. and Pankow, G. W. 1936 *Helv. Chim. Acta*, **19**, 930.
Misch, L. and Picken, L. 1937 *Z. phys. Chem.* B. (In the Press.)
Pochin, E. 1935 Private communication from the author (1936).
Polanyi, M. 1925 *Z. tech. Phys.* **6**, 122.
Saxl, P. 1907 *Beitr. chem. Physiol. Path.* **9**, 1.
Wöhlisch, E. 1926 *Z. Biol.* **85**, 379.
— 1932 *Ergebn. Physiol.* **34**, 406.
Wöhlisch, E. and Clamann, H. G. 1931 *Z. Biol.* **91**, 399.

DESCRIPTION OF PLATE 3

All photographs taken with Cu radiation filtered with 0·02 mm. Ni foil, using a pin-hole collimator 1·0 × 60 mm. Exposure time = 1½ hr. Tube operating at 30 kV, 17 mA.

FIG. 9—Anterior byssal retractor muscle of *Mytilus edulis*, *ca.* 40 % stretch, temperature *ca.* 15° C. Distance from muscle to film 27·8 mm.

FIG. 10—A small bundle of fibres from this muscle photographed in the contracted condition under zero load. Distance from muscle to film 25·0 mm.

FIG. 11—The same muscle dried under tension, washed in distilled water and redried under tension. Distance from muscle to film 31·1 mm.

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Olfactory Conditioning in a Parasitic Insect and its Relation to the Problem of Host Selection

BY W. H. THORPE, M.A., PH.D. AND F. G. W. JONES, B.A.
From the Department of Entomology, Zoological Laboratory, Cambridge

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INTRODUCTION

It has often been stated that insect species which breed on two or more hosts tend to become split into biological races, each of which is attached to a particular food-plant or host as the case may be. But although the fact of the existence of biological races is well known, their mode of origin is, in most cases, in dispute. It is not known how far the biological characters of such races are germinally fixed, and it has been suggested that in many cases a group of individuals may become restricted to certain host species not by any germinal change, but by a kind of conditioning, as a result of which the adult female is attracted for oviposition to the particular species on which she had fed as a larva. This is the idea behind the so-called "Host Selection Principle" of Walsh, and it is a possibility which must be carefully considered when assessing the value of experiments attempting to establish the inheritance of acquired characters. It is obvious that if a conditioning of this sort was actually occurring, a polyphagous insect species might rapidly become split into two or more populations each attached to a given species of host (plant or animal as the case may be). The barrier thus created might persist for a very long time without any hereditary specialization taking place. It is known, however, that the characters which distinguish biological races are in fact often germinally fixed, and in some instances it has been shown that even though all structural differences are absent there may be repugnance for cross-breeding between two biological races of the same species (Thorpe 1930*b*). It is nevertheless often difficult to visualize the means by which such germinal differences could become established without the aid of the ecological separation which such host conditioning might provide.

Although from the evolutionary point of view the postulation of such "pre-imaginal conditioning" would seem to be reasonable enough, yet there are obvious theoretical physiological difficulties in the way. The subject of reflex conditioning in insects has as yet barely been touched, but it is known that in some insects where conditioning is possible (ants, hive bees and butterflies) the effect is only of short duration. It might seem somewhat surprising therefore if in others conditioning could endure throughout a pupal period of considerable length. Another obvious difficulty is that involved in explaining how such conditioning could persist in the nervous system during the pupal period at a time when great structural reorganization is going on. It is possible, however, that this objection is more apparent than real, since there is not in general the same sudden and extensive histolysis in the nervous system that one finds in many other tissues. Indeed, in many insects the nervous system of the larva is transformed into that of the adult by a gradual series of changes, often commencing well before pupation. Since the subject is of such practical and theoretical interest it is somewhat surprising to find that there is little work which throws any light upon the problem. In 1929 the senior author published an account of the host plant races of the small ermine moth (*Hyponomeuta*). This work gave some indication that although the oviposition responses of the moths were germinally fixed yet rearing on an unaccustomed food plant did have some modifying effect. The figures, although suggestive were not, however, fully significant on this particular point. Owing chiefly to the low reproductive rate which made *Hyponomeuta* very unsatisfactory for this type of work, the experiments were not continued. Larson's experiments (1927) on the oviposition responses of the pea weevil, *Bruchus quadrimaculatus*, led him to the conclusion that the adults of this species show no marked predilection for the host in which they have bred, and that continued breeding in a given host does not appear to intensify the preference for that host. It may, however, be objected that a highly polyphagous insect such as *Bruchus* is not one in which the effect might be expected to manifest itself particularly strongly, and that it would be more reasonable to look for it in an insect attacking a few hosts only. In this connexion the work of Thompson and Parker (1928) and that of Quayle (1926) is more convincing. The former authors, in the course of extensive work on the European corn-borer *Pyrausta nubilalis*, failed to show that individuals reared from maize were biologically differentiated from those on the original host plant *Artemisia*, except that the slight positive predilection which *Artemisia* individuals have for oviposition on that plant seems to have been lost by the maize individuals. Quayle worked with the codling moth *Carpocapsa pomonella*. His experiments, not completely conclusive, failed to show that the curious habit of this insect of attacking walnuts in California was associated with the development of a biological race.

It is natural to expect that a pre-imaginal conditioning effect would be more likely to develop in the case of endoparasitic insects since the larvae of such forms are in particularly close and intimate relationship with their hosts. It seems that the only parasite in which the matter has been at all adequately investigated is that of the egg parasite *Trichogramma*. In this form Salt (1935) has failed to find any evidence of preference for eggs of *Sitotroga* in those individuals reared on that species. This, however, is not surprising since Salt has since shown that the olfactory sense plays little or no part in the host finding of *Trichogramma*. It seems very probable that *Trichogramma* is exceptional among endoparasites in this respect, and therefore it seemed worth while investigating the subject in some other parasitic hymenopteron, preferably one which is restricted to a few host species and in which the finding of the host is primarily by the olfactory sense. For this purpose *Nemeritis canescens* (Grav.) seemed almost ideal. *Nemeritis* is an ichneumonid parasite which develops at the expense of the flour moths *Ephestia kühniella* (Zell.) and *E. elutella* (Hb.) (Family Pyralidae, subfamily Phycitinae). It is very easily reared in the laboratory and completes its life history in the relatively short period of 3–4 weeks at optimum temperature; moreover it is completely parthenogenetic, males being unknown in this country. A culture of it has been reared in this Department continuously during the last 3 years. Under natural conditions in Europe it is known only from the two species of *Ephestia* above mentioned, although in the U.S.A. a race exists which while structurally identical develops solely at the expense of the large wax moth (*Galleria* *mellonella* L.) also of the Family Pyralidae but belonging to the subfamily Galleriinae (Richmond 1925). In the course of experimental work which it is hoped to publish before long, the senior author discovered that the European race of *Nemeritis canescens* could be reared without great difficulty upon the small wax moth *Meliphora grisella* (F.), a closely related insect belonging to the same subfamily. Although under normal conditions oviposition could only very rarely be obtained upon this host, yet it was found that by contaminating the larvae of *Meliphora* with the smell of the normal host in various ways egg-laying could fairly easily be secured. The egg is placed in the blood of the host and the larva develops in the usual manner, first feeding on the blood and later on the fat body and other tissues, ultimately emerging from the skin of the host in order to spin its cocoon. Repeated attempts were made to rear it on *Galleria* but only on a few occasions were these attended with success. It appears that even when oviposition of the European race can be secured on this host only a small proportion of the eggs are able to develop.

Although it is not necessary to describe here in great detail the way in which *Nemeritis* finds its host, yet the subject must be dealt with briefly in order to establish with certainty that smell is the most important factor in the choice. One could not, of course, expect a conditioning of this type to be concerned with anything except chemical sense. It was shown by means of an olfactometer of the McIndoo type that the insect is strongly attracted by odour* to the region in which the host is to be found. The possibility that it is also attracted by the floury substance on which the host feeds was also investigated, and the results of this experiment are shown in Table I. Having reached the region in which the host is situated the parasite commences to walk about exploring the surroundings by means of its antennae, which are in constant vibration. It is immediately stimulated by any traces of the host larva, and extrusion of the ovipositor is at once produced if the parasite walks over a surface which an *Ephestia* larva has previously traversed. This is clearly a case of the "Kontakt-Geruchsinn" described by Murr (1930). This effect will last for a considerable time, and it is possible to produce immediate unsheathing of the ovipositor of *Nemeritis* merely by placing it in a box in which an *Ephestia* larva had been confined for an hour or two more than a week before. As soon as the ovipositor is unsheathed the insect proceeds to stab with it more or less at random, although the stabbing is more in evidence when the animal is in the corner of the box or cage, when there are crevices in the substratum or when it is on some fairly soft or easily penetrable substance such as muslin. The extrusion of the ovipositor and subsequent stabbing is a very good indication of stimulation by "Kontakt-Geruchsinn". But it can be induced by other methods, for instance stabbing takes place much more readily in insects which have been kept for a long time without access to any host. Dissection of such animals shows that the oviducts are tightly packed with eggs, and it may be that this distention enables the stabbing reflex to be more easily called forth. In insects kept under these conditions we have observed occasional stabbing through the muslin of the cage even without any traces of the host being present. Stabbing with the ovipositor is also to some extent a defence mechanism, for if the insect be seized with a pair of forceps or by the fingers it stabs vigorously until released. Similarly if the *Nemeritis* are kept under crowded conditions in the cages, owing to their constant jostling against one another in their attempts to get food, they are frequently seen to stab one another, and on two occasions we have dissected female *Nemeritis* which contained a dead first-stage larva in the body cavity. It is fairly certain that this was a case of parasitism resulting from defensive stabbing while in a crowded cage.

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* Similar conclusions were arrived at by von Stein-Beling (1934) as a result of experiments with marked individuals.

Finally the texture of the substratum may itself play a part in inducing the unsheathing of the ovipositor, and in the case of insects which have been kept for a long period without previous chance of oviposition, it is not difficult to elicit a tentative stabbing of the cocoon of *Meliphora*. From experiments described below it is clear that this is mainly a response to the texture of the cocoon and not to the odour of the host. Once the stabbing reflex has been called forth it is evident that some perception of the texture of the host, and possibly also of size, comes into play. Hosts which are too large or too small are not accepted even though they are very strongly contaminated with the odour of *Ephestia*. Nor has it so far been found possible to secure oviposition in dead hosts although these are readily stabbed. Apparently movement of the host upon pricking is the final stimulus which induces the passage of the egg down the ovipositor.

It seems that sight plays little or no part in the finding of the host, since oviposition experiments conducted in darkness yielded as high a rate of parasitism as those in the light. It appears also that the olfactory sense resides entirely in the antennae, for it is a simple matter to amputate the antennae leaving only the basal segments. Recovery from the operation is rapid, and in cages the behaviour of insects thus treated appears normal, save that the movements are somewhat more jerky and erratic than usual. There is no difficulty in flying. When they are transferred to a cage containing oatmeal contaminated with *Ephestia* larvae they show no reaction whatever, displaying no interest in the larvae whether naked or in their cocoon. Operated insects feed and live apparently normally, and the cleaning reflexes of the abdomen and of the appendages remain unaffected.

Further evidence of the unimportance of visual sense in finding the host is shown by the lack of response to objects such as gelatine capsules covered with muslin approximately the same shape and size as the host cocoon. As long as there is no trace of host odour attached to these objects the insect betrays no interest in them at all. Moreover under natural conditions the host, at the stage when it is being attacked by the parasite, is usually completely covered by the flour and is entirely hidden from view.

It will be seen from this brief account that the host selection of *Nemeritis* closely resembles that of *Microplectron fuscipennis* (Zett.) as described by Ullyett (1936). It is, however, clear that in *Nemeritis* the sense of smell plays an even more important part than it does in the case of *Microplectron*, since vision appears to play no part at all and since oviposition can be obtained almost as easily in a naked larva as in the larva enclosed in the cocoon, provided the correct odour is present. It appears, therefore, that *Nemeritis* is a particularly suitable insect for the present investigation.

METHODS

As has already been indicated, an extensive series of preliminary experiments, details of which it is not necessary to enter into here, had clearly shown odour to be the dominant factor in host selection. Accordingly some experimental method was required which would permit of investigation of the responses to this factor alone. After a number of trials a simple olfactometer, differing in various small points from that originally described by McIndoo (1926) was found to be highly satisfactory (fig. 1).

The insects to be investigated are placed in the tube *A* and are allowed to settle down, the clip *B* being closed. The larvae, which are to be the source of the test odour, are placed in one or other of the tubes *GG*. They are prevented from escaping by a plug of cotton-wool at one end of the tube and a cap of bolting silk at the other. Air is then drawn through the tube *D* by means of a suction pump, the flow through the two tubes *HH* being equalized by means of a system of clips and wash-bottles not shown in the illustration. When a steady flow has been established the clip *B* is opened so that the insects in *A* can make their way towards the light *L* from which they have been protected by the screen *S*. If any insects find difficulty in leaving *A*, the space in which they are enclosed is gradually reduced by the plunger *P* until all have passed through. On reaching the

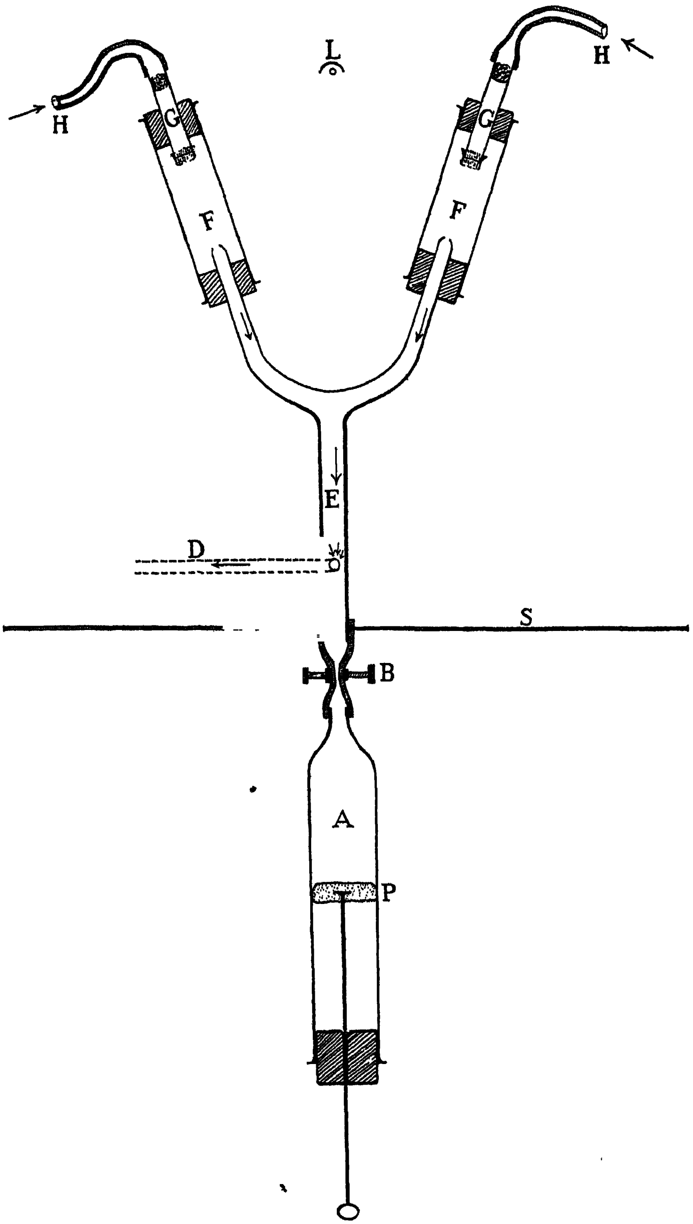

FIG. 1—Diagram of olfactometer. Arrows indicate direction of air flow. For explanation see text

fork of the glass tube *E* they are exposed to the alternative odours and having made their response are eventually trapped in one or other of the tubes *FF*.

Preliminary tests showed the region of contact of the two air streams to be sharply defined, thus facilitating the making of a genuine response. Experiments in which the apparatus was run without any "bait" showed that there was no appreciable bias towards one side, but in order to avoid any possible error due to this source the position of the "baits" was alternated from one arm to the other at frequent intervals during each series of experiments. Thus in one half of each set the main stimulus would be on the right limb and in the other half on the left. After every few experiments the apparatus was washed out with a stream of water and dried with alcohol followed by a current of air. This was thought advisable in order to obviate the possibility of errors due to accumulation of dust from the host larvae, or to the traces left by the *Nemeritis* themselves. As a matter of fact some test experiments designed to show the influence on the results of these factors failed to give any indication that they were appreciable sources of error. The apparatus worked well provided that the temperature was maintained constant within certain limits. If the temperature is too low the insects are sluggish and will not go through the apparatus at all; if it is too high they tend to rush through so rapidly that it is impossible for them to exercise any "choice" and the resulting distribution is random. In actual fact the great majority of the experiments were carried out in a constant temperature room at 25° C. and 70 % relative humidity. This was found to give practically the optimum rate of passage.

With few exceptions the *Nemeritis* used throughout the experiments had not had any previous opportunity for oviposition. They were for the most part between three and ten days old and were always kept well supplied with food in the form of water, raisins or honey. From this it may be assumed that the results of the olfactometer experiments were all indicative of oviposition response, not feeding response.

RESULTS OF EXPERIMENTS

a—Nemeritis *reared on* Ephestia

As a first experiment it was desirable to determine whether one of the normal food materials of the host, namely oatmeal, without any traces of the *Ephestia* itself exercised any attraction for *Nemeritis*, certain chance observations having indicated that this might be the case. Accordingly a group of fifty *Nemeritis* reared from the normal host *Ephestia* were put through the olfactometer, one arm of which contained clean oatmeal, the other arm being empty. The results of these experiments are shown in Table I. It will be seen that out of a total of 402 choices the arm containing the oatmeal was favoured 235 times or 58·5 % and the empty arm 167 times.

TABLE I—RESPONSES OF FIFTY INDIVIDUALS OF *NEMERITIS CANESCENS* (REARED FROM *EPHESTIA*) TO CLEAN OATMEAL IN OLFACTOMETER. (FOR FULLER EXPLANATION SEE TEXT)

	a	*a′*	*n*		
Exp.	Oats	Blank	Total	*p*	*ap*
1	9	11	20	0·45	4·05
2	12	15	17	0·706	8·47
3	8	12	20	0·40	3·2
4	11	5	16	0·69	7·59
5	8	9	17	0·47	3·76
6	7	5	12	0·58	4·06
7	11	10	21	0·524	5·75
8	8	5	13	0·615	4·92
9	10	4	14	0·71	7·1
10	8	4	12	0·666	5·28
11	7	3	11	0·636	4·45
12	6	4	10	0·60	3·6
13	5	3	8	0·62	3·1
14	5	4	9	0·55	2·75
15	4	5	9	0·445	1·78
16	13	5	18	0·725	9·42
17	15	7	22	0·68	10·20
18	9	9	18	0·5	4·5
19	8	10	18	0·44	3·52
20	9	7	16	0·56	5·04
21	8	3	11	0·72	5·76
22	5	2	7	0·71	3·55
23	7	4	11	0·636	4·45
24	11	5	16	0·687	7·55
25	14	7	21	0·666	9·32
26	11	7	18	0·61	6·71
27	6	2	8	0·75	4·5
Total	235	167	402		

= Oats 58·5 %; = Blank 41·5 %.

$$\text{Standard error} = \sqrt{\frac{pq}{n}} = \pm 2{\cdot}49\,\% \text{ when } p = 0{\cdot}5 \text{ and } q = 0{\cdot}5.$$

$$\bar{p} = \tfrac{235}{402} = 0{\cdot}585, \quad \bar{q} = 1 - \bar{p} = 0{\cdot}415.$$

$$\chi^2 = \frac{\Sigma\,(ap) - n\bar{p}}{\bar{p}\bar{q}} = \frac{144{\cdot}38 - (235 \times 0{\cdot}585)}{0{\cdot}585 \times 0{\cdot}415} = \frac{6{\cdot}78}{0{\cdot}243} = 27{\cdot}9, \quad \therefore P = 0{\cdot}36.$$

The standard error calculated from the formula $\sqrt{\frac{pq}{n}}$ for an expected ratio of 1 : 1, i.e. when p and q are each 0·5, is ± 2·49 %; and since the value 58·5 % exceeds 50 % by more than twice the standard error the result can be considered as significant. In all the subsequent tables also the S.E. has been calculated on the basis $p = 0{\cdot}5$. The tables of Purewal and Rao (1936) have been found convenient for this purpose and have saved considerable labour.

It will be seen from a glance at the figures in Table I that there is a considerable variation in the results of the individual experiments, extremes being Exps. 3 and 27. Accordingly it seemed desirable to test the homogeneity of the series of experiments by means of the formula $\chi^2 = \frac{\Sigma(ap) - n\bar{p}}{\bar{p}\bar{q}}$ where a = the number of "choices" for oats in each experiment, $p = a$, expressed as a proportion of the total number for the experiment; n = the sum of the totals for oats for the whole series, $\bar{p} = n$, expressed as a proportion of the total number for all experiments, and $\bar{q} = 1 - \bar{p}$. The problem is to determine whether the variance shown by the series of values is in agreement with the expectation of normal variance; or in other words can the variations be considered as due to chance alone or are they evidence of some heterogeneity in the behaviour of the insects or in the conditions of experiment? Using the table of χ^2 given by Fisher (1936) it will be seen that when the number of degrees of freedom (i.e. one less than the number of experiments in the series) is 26 the value of χ^2 corresponding to a probability (P) of 0·05, is 38·88. Therefore any value of χ^2 *below* this figure is satisfactory in that it means that the data are compatible with a hypothesis of homogeneity. Actually it will be seen that the value found for χ^2 of 27·9 corresponds to a P of approximately 0·36. In other words the probability that a χ^2 of 27·9 should be exceeded is more than 1 in 3; therefore we can be satisfied that the results of the series of experiments are consistent. From the series we can therefore conclude that the smell of fresh oatmeal has a slight attraction for *Nemeritis canescens*.

The next point was to determine the intensity of attraction exerted by the scent of larvae of *Ephestia* on *Nemeritis* reared upon this, their normal, host. For this purpose six half-grown living larvae of *Ephestia* were placed in one arm of the olfactometer, the other being left blank. The results of this set of experiments in which seventy individuals of the parasite were employed are given in Table II. It will be seen that there is a very strong preference for the arm containing the larvae; since the standard error is only ± 3·43 % the results are obviously significant. χ^2, calculated as described above, also gives a result which is entirely satisfactory in that it is much less than the critical value of 16·9 which corresponds in Fisher's tables to a p of 0·05 when $n = 9$. Actually a χ^2 of 8·05 indicates a P of approximately 0·47.

TABLE II—RESPONSES OF SEVENTY INDIVIDUALS OF *NEMERITIS CANESCENS* (REARED FROM *EPHESTIA*) TO LARVAE OF *EPHESTIA* IN OLFACTOMETER

Exp.	*Ephestia*	Blank	Total
1	15	0	15
2	23	5	28
3	13	2	15
4	7	0	7
5	18	2	20
6	24	1	25
7	21	5	26
8	18	4	22
9	12	3	15
10	35	5	40
Total	186	27	213

= *Ephestia* 87·3 %; = Blank 12·7 %; standard error ± 3·43 %.

$$\chi^2 = \frac{\Sigma\,(ap) - n\bar{p}}{\bar{p}\bar{q}} = 8{\cdot}05, \quad \therefore\ P = 0{\cdot}47 \text{ approx.}$$

Following this result the reaction of *Ephestia*-reared *Nemeritis* to a choice of the two hosts was investigated; six larvae of *Meliphora* being placed in one arm of the apparatus and six of *Ephestia* in the other. Care was taken to use clean larvae of approximately the same size and age in each case and most careful precautions were taken to prevent the contamination of one of the hosts by the smell of the other. The results of a series of experiments using thirty-five individuals are given in Table III. It will be seen that there is no evidence that the odour of *Meliphora* larvae exerts any attraction for *Nemeritis* and allowing for the standard error and comparing Tables II and III it is obvious that there is no significant difference. This is borne out by consideration of Table IV which presents the results of a set of experiments in which the alternative was between *Meliphora* and "blank". The figures 50·8 and 49·2 % do not differ significantly from 50 % and the results therefore can be ascribed to chance. A glance at the sets of figures in Tables III and IV shows that they are remarkably homogeneous, consequently it was not considered necessary to evaluate χ^2.

From these results therefore we may conclude that *Nemeritis* reared on its normal host *Ephestia*, while responding very strongly to the smell of

TABLE III—RESPONSES OF THIRTY-FIVE INDIVIDUALS OF *NEMERITIS CANESCENS* (REARED FROM *EPHESTIA*) TO LARVAE OF *EPHESTIA* AND *MELIPHORA* IN OLFACTOMETER

Exp.	*Ephestia*	*Meliphora*	Total
1	12	6	18
2	6	0	6
3	10	1	11
4	9	3	12
5	20	3	23
6	18	3	21
7	8	1	9
8	7	1	8
9	9	3	12
10	8	3	11
11	18	2	20
12	29	1	30
Total	154	27	181

= *Ephestia* 85 %; = *Meliphora* 15 %; standard error ± 3·72 %.

TABLE IV—RESPONSES OF 150 INDIVIDUALS OF *NEMERITIS CANESCENS* (REARED FROM *EPHESTIA*) TO LARVAE OF *MELIPHORA* IN OLFACTOMETER

Exp.	*Meliphora*	Blank	Total
1	7	7	14
2	7	17	24
3	2	7	9
4	16	10	26
5	9	4	13
6	8	7	15
7	4	6	10
8	7	7	14
9	7	6	13
10	7	13	20
11	11	8	19
12	4	6	10
13	2	8	10
14	5	5	10
15	8	2	10
16	6	4	10
17	3	7	10
18	3	7	10
19	7	3	10
20	7	3	10
21	6	4	10
22	8	2	10
23	8	2	10
24	9	8	17
25	8	6	14
26	2	3	5
27	2	8	10
28	10	6	16
29	1	2	3
Total	184	178	362

= *Meliphora* 50·8 %; = Blank 49·2 %; standard error ± 2·63 %.

Ephestia, is not significantly attracted by the odour of *Meliphora*. The occasional instances of oviposition in the larvae of *Meliphora* by insects caged with them (see introductory section) must therefore be due to stimulation of senses other than the olfactory.

b—Nemeritis *reared on* Meliphora

Concurrently with the above series of experiments a similar series was made, using instead *Nemeritis* which had been reared upon *Meliphora*. The insects used, unless otherwise stated, were between 1 and 2 weeks old. Oviposition was secured by contaminating the larvae of *Meliphora* with the smell of *Ephestia* before presenting them to the parasite. After parasitization they were returned to their normal milieu of broken honeycomb. The

TABLE V—RESPONSES OF APPROXIMATELY SEVENTY INDIVIDUALS OF *NEMERITIS CANESCENS* (REARED FROM *MELIPHORA*) TO LARVAE OF *EPHESTIA* IN OLFACTOMETER

Exp.	*Ephestia*	Blank	Total
1	8	2	10
2	9	1	10
3	8	2	10
4	8	2	10
5	9	1	10
6	9	1	10
7	10	0	10
8	9	1	10
9	9	1	10
10	7	3	10
11	8	2	10
12	6	1	7
13	8	0	8
14	8	0	8
15	7	2	9
16	8	1	9
17	6	1	7
18	6	1	7
19	16	5	21
20	23	3	26
21	18	2	20
22	16	4	20
23	17	3	20
24	14	5	19
25	12	4	16
26	14	2	16
27	13	3	16
28	12	2	14
Total	298	55	353

= *Ephestia* 84·4 %; = Blank 15·6 %; standard error ± 2·66 %.

Nemeritis which emerged were carefully isolated from the odour of *Ephestia,* and except in a few instances had not had any opportunity for oviposition before being used for the experiments. Tables V, VI and VII correspond to Tables II, III and IV. It will be seen at a glance that there is respectively a very considerable difference between them and the question arises whether these differences are significant. Consider first the differences between the results given in Table II and those in Table V. In each case the alternatives are *Ephestia* and "blank", but in one instance the parasites employed were reared on *Ephestia,* in the other on *Meliphora.* In a com-

TABLE VI—RESPONSES OF 112 INDIVIDUALS OF *NEMERITIS CANESCENS* (REARED FROM *MELIPHORA*) TO LARVAE OF *EPHESTIA* AND *MELIPHORA* IN OLFACTOMETER

Exp.	*Ephestia*	*Meliphora*	Total	
1	13	5	18	1st generation
2	13	7	20	
3	16	5	21	
4	13	6	19	
5	15	4	19	
6	10	6	16	
7	20	12	32	
8	10	6	16	
9	16	3	19	4th generation
10	12	3	15	
11	14	6	20	5th generation
12	13	9	22	7th generation
13	13	9	22	
14	16	4	20	
15	17	3	20	
16	18	6	24	
17	13	6	19	
18	13	7	20	
19	23	5	28	
20	8	10	18	
21	8	20	28	
22	9	5	14	
23	14	12	26	8th generation
24	16	9	25	
25	14	11	25	
26	13	8	21	
27	15	9	24	
28	14	6	20	
Total	389	202	591	

= *Ephestia* 65·8 %; = *Meliphora* 34·2 %; standard error ± 2·06 %.

$$\chi^2 = \frac{\Sigma\,(ap) - n\bar{p}}{\overline{pq}} = 38\cdot5, \quad \therefore P = 0\cdot06 \text{ approx.}$$

parison of this sort the χ^2 distribution is again of value, the method being employed as a test of independence (see Fisher 1936, p. 90). Table XIII shows the details of the process of calculating χ^2 from the formula

$$\chi^2 = \frac{(ad-bc)^2 N}{(a+b)(c+d)(a+c)(b+d)}.$$

In this case, since there is only one "degree of freedom" the values for χ^2 in Fisher's table must be read from the column $n = 1$. In this column the value 3·841 corresponds with the value of $P = 0{\cdot}05$. Thus a value of χ^2 *greater* than 3·841 implies that there is less than 1 chance in 20 that the two classifications are independent. Since in the example under discussion $\chi^2 = 9{\cdot}13$ we can safely assume that the differences between the two sets of tables are significant. For one degree of freedom a χ^2 of 9·13 corresponds to a P of less than 0·01; i.e. the probability that the differences are due to chance is less than 1 in 100. One is consequently justified in assuming that the rearing of *Nemeritis* on *Meliphora* causes a real difference in the intensity of its response to the smell of *Ephestia*.

But such a result might merely indicate a general loss in vitality consequent upon rearing on an abnormal host, resulting in a decrease of the vigour with which the host is sought. That this is not the true explanation is shown when we come to compare the results of tests in which the alternatives were *Ephestia-Meliphora* and *Meliphora*-"blank". In the case of the *Ephestia-Meliphora* alternative a comparison of the results of Table III with those of Table VI as above gives a χ^2 of 25·3 which again greatly exceeds the critical value of 3·841 and corresponds to a P of *much* less than 1 in 100, similarly a comparison of the results of Tables IV and VII gives $\chi^2 = 15{\cdot}3$ which again implies a P of less than 0·01. From these results we are accordingly justified in concluding that rearing upon *Meliphora* results not merely in a slight decrease in the intensity of the response of the adult parasites to *Ephestia*, but that it also endows them with a marked tendency to respond to the smell of *Meliphora*, whether they encounter it alone or in competition with that of *Ephestia*.

c—Nemeritis *reared on* Ephestia *but "conditioned" to* Meliphora

The effect of an abnormal host on the olfactory responses of the adult parasite having been thus established, the question at once arises whether the result is due to the influence of the environment of the larval stage lasting into the adult life or to a conditioning of the adult immediately upon its emergence from the pupa. The second alternative appeared very

TABLE VII—RESPONSES OF 112 INDIVIDUALS OF *NEMERITIS CANESCENS* (REARED FROM *MELIPHORA*) TO LARVAE OF *MELIPHORA* IN OLFACTOMETER

Exp.	*Meliphora*	Blank	Total	
1	4	4	8	1st generation
2	4	4	8	
3	7	3	10	
4	5	5	10	
5	14	5	19	
6	18	3	21	
7	14	7	21	
8	3	7	10	4th generation
9	7	3	10	
10	8	2	10	
11	6	4	10	
12	7	3	10	
13	4	6	10	
14	7	3	10	
15	9	1	10	
16	8	2	10	
17	10	0	10	
18	4	6	10	
19	8	2	10	
20	6	4	10	5th generation
21	6	4	10	
22	7	3	10	
23	8	2	10	
24	11	10	21	8th generation
25	11	8	19	
Total	196	101	297	

= *Meliphora* 66 %; = Blank 34 %; standard error ± 2·9 %.

$$\chi^2 = \frac{\Sigma(ap) - n\bar{p}}{\bar{p}\bar{q}} = 33, \quad \therefore P = 0{\cdot}10.$$

plausible, because of the fact that the adult *Nemeritis* remains in its cocoon, which itself usually includes part of the skin of the host, for 1 or 2 days after emergence from the pupa.

In order to test the possibility of conditioning after emergence from the cocoon the following experiment was performed. Two sets of parasites were isolated immediately on emergence from their normal host and were then confined for a period of 7–10 days in a jar containing crumbled honeycomb densely infested with larvae and pupae of *Meliphora*. They were then put through the olfactometer with the result shown in Tables VIII and IX. Since the results of the individual experiments were obviously reasonably consistent the χ^2 test was not applied.

Comparing the figures given in Table VIII with those obtained for "unconditioned" parasites from *Ephestia* given the same alternatives

(Table IV), it will be seen that whereas the normal *Ephestia*-reared insects show no significant tendency to choose the arm containing *Meliphora* in preference to the empty arm, 61·5 % of the "conditioned" insects chose the *Meliphora* side. The application of the formula

$$\chi^2 = \frac{(ad-bc)^2 N}{(a+b)(c+d)(a+c)(b+d)}$$

to this pair of results gives the figure 7·33 which again corresponds to $P < 0{\cdot}01$, which, as pointed out above, is entirely satisfactory. When, however, we come to contrast the results of Tables III and IX we see that there is not this clear-cut difference, and as might be anticipated χ^2 works out at a figure, 0·24 corresponding to a P of 0·6, which shows that the difference between the two is not significant.

TABLE VIII—RESPONSES OF FORTY-FIVE INDIVIDUALS OF *NEMERITIS CANESCENS* (REARED FROM *EPHESTIA* BUT "CONDITIONED" TO *MELIPHORA* LARVAE FOR 7 DAYS IMMEDIATELY UPON EMERGENCE) TO LARVAE OF *MELIPHORA* IN OLFACTOMETER

Exp.	*Meliphora*	Blank	Total
1	9	7	16
2	9	2	11
3	4	3	7
4	7	5	12
5	8	6	14
6	4	2	6
7	6	2	8
8	5	7	12
9	5	5	10
10	8	6	14
11	6	4	10
12	5	6	11
13	5	8	13
14	10	1	11
15	4	5	9
16	4	5	9
17	9	6	15
18	11	0	11
19	6	4	10
20	6	5	11
21	9	3	12
22	9	1	10
23	8	4	12
24	7	5	12
Total	164	102	266

= *Meliphora* 61·6 %; = Blank 38·4 %; standard error ± 3·06 %.

We can conclude therefore that caging the insects with the false host *Meliphora* in this way does result in a tendency to seek out that particular host, but that this tendency is only manifest when the normal host is absent; when the smell of *Ephestia* is also present its attraction proves overwhelming. But it will be seen by comparing Table VIII with Table VII and Table IX with Table VI that there is a difference in behaviour between *Nemeritis* which have been reared on *Meliphora* and those which have been merely conditioned to it after emergence from the cocoon. Here again, as we might expect, when *Meliphora* only is offered, the conditioned *Nemeritis* are almost as efficient in their response to it as are those which have been actually reared on that host; indeed χ^2 as between series VII and VIII is only 1·15, which indicates that the differences are not actually significant. On the other hand, when both hosts are offered (series VI and IX) there is a big difference in the results, showing that in this case rearing on the

TABLE IX—RESPONSES OF FIFTY INDIVIDUALS OF *NEMERITIS CANESCENS* (REARED FROM *EPHESTIA* BUT "CONDITIONED" TO *MELIPHORA* LARVAE FOR 7 DAYS IMMEDIATELY UPON EMERGENCE) TO LARVAE OF *EPHESTIA* AND *MELIPHORA* IN OLFACTOMETER

Exp.	*Ephestia*	*Meliphora*	Total
1	16	0	16
2	16	0	16
3	10	9	19
4	14	0	14
5	10	0	10
6	13	3	16
7	11	1	12
8	12	2	14
9	8	2	10
10	9	1	10
11	6	1	7
12	7	3	10
13	10	0	10
14	8	2	10
15	16	4	20
16	9	3	12
17	8	4	12
18	9	1	10
19	8	2	10
20	10	2	12
21	10	4	14
Total	220	44	264

= *Ephestia* 83·4 %; = *Meliphora* 16·6 %; standard error ± 3·08 %.

Meliphora is much more efficient in producing a tendency to respond to it than is adult conditioning alone.

These experiments show, therefore, that while some conditioning is possible during the adult stage there is also an additional effect which may be either due to the influence of the environment on the adult during the quiescent period in the cocoon immediately upon emergence, or else to environmental influence exerted through the larva.

d—Nemeritis *reared on* Meliphora *but subsequently "de-conditioned" by contact with* Ephestia

To test which of these two alternatives is the true one is not so easy as might at first be supposed. It can be attempted in two ways. First, the insects might be dissected from their cocoons while in the early pupal stage and reared in gelatine capsules away from any host influence. Secondly, the parasitized *Meliphora* larvae can be transferred, as soon as they have finished feeding, to meal thoroughly contaminated with the cocoons and faeces of *Ephestia* so that the parasite larvae emerging from their hosts will be surrounded by the influence of *Ephestia*, and will thus become as it were "de-conditioned". This method is open to the obvious objection that since the cocoon of the parasite often includes silk spun by the host larva as well as remnants of the host skin the parasite during the period of its pupal and earliest adult life is not subjected to the smell of *Ephestia* only but in all probability to a mixture of the two with that of *Ephestia* greatly preponderating. Since, however, *Ephestia* is the normal host, for which the parasite initially has a very strong preference, and since, as experiments described above have shown that unconditioned *Nemeritis* make no distinction between *Meliphora* and its absence, it does not appear that the objection is a very serious one.

The first of these methods, although obviously the most satisfactory in many ways, has unfortunately not proved feasible. Many ichneumonid parasites can be removed from their cocoons in the pupal stage without injury and can subsequently be reared without any great mortality. But the pupa of *Nemeritis* is soft and very susceptible to injury, and attempts to carry out the experiment have so far come to nought owing to the high mortality. Consequently the alternative method has been adopted with the results shown in Tables X and XI. It is convenient to term this process "de-conditioning" on the assumption that rearing on *Meliphora* will tend to condition the parasite to that host and that exposure on emergence to the normal host will counteract the effect and so produce "de-conditioning".

TABLE X—RESPONSES TO LARVAE OF *MELIPHORA* AND *EPHESTIA* OF 102 INDIVIDUALS OF *NEMERITIS* REARED ON *MELIPHORA* BUT SUBSEQUENTLY "DE-CONDITIONED" BY CONTACT WITH *EPHESTIA*

Exp.	*Ephestia*	*Meliphora*	Total
1	18	2	20
2	19	1	20
3	9	1	10
4	15	4	19
5	17	2	19
6	16	3	19
7	16	3	19
8	17	1	18
9	14	6	20
10	18	2	20
11	18	1	19
12	11	9	20
13	16	4	20
14	18	1	19
15	16	0	16
16	12	9	21
17	14	10	24
18	13	11	24
19	8	9	17
20	18	4	22
21	15	8	23
22	16	7	23
23	15	7	22
24	16	8	24
25	12	9	21
26	13	9	22
27	14	7	21
28	12	8	20
29	26	9	35
30	21	4	25
31	18	4	22
Total	481	163	644

= *Ephestia* 74·8 %; = *Meliphora* 25·2 %; standard error ± 1·97 %.

Comparing the results obtained with these "de-conditioned" insects with those from other sets of experiments, we notice that whereas insects from *Ephestia* (Table III), given *Ephestia* and *Meliphora* as alternatives, chose the former host in 85 % of cases, those listed in Table X went to the *Ephestia* arm in 74·8 % only. χ^2 for this pair of results works out at 8·61 so we can regard the difference as significant and conclude that while "de-conditioning" reduces the effect of the rearing on *Meliphora* it does not wipe it out entirely, there being a kind of residual effect due apparently to influences acting during the period of larval life. Similarly comparing series VI with series X we obtain $\chi^2 = 11{\cdot}66$, showing that there is a significant difference between the two sets and that "de-conditioning" does not entirely remove the effect of the larval environment.

TABLE XI—RESPONSES TO LARVAE OF *MELIPHORA* OF FIFTY INDIVIDUALS OF *NEMERITIS* REARED ON *MELIPHORA* BUT SUBSEQUENTLY "DE-CONDITIONED" BY CONTACT WITH *EPHESTIA*

Exp.	*Meliphora*	Blank	Total
1	16	4	20
2	15	5	20
3	17	3	20
4	14	6	20
5	12	5	17
6	13	6	19
7	15	5	20
8	13	7	20
9	17	3	20
10	16	6	22
11	22	3	25
12	8	1	9
13	23	8	31
14	18	7	25
15	21	9	30
16	18	9	27
17	17	13	30
Total	275	100	375

= *Meliphora* 73·4 %; = Blank 26·6 %; standard error ± 2·58 %.

It will be seen that just as when dealing with simple conditioning, so with "de-conditioned" insects, there is a difference in the results depending on whether both hosts are offered in the olfactometer or only one. A comparison of series VII and XI shows that "de-conditioning" in the case of the alternatives *Meliphora*-"blank" has no effect at all in reducing the influence of the larval environment. In fact it so happens that the "de-conditioned" insects have given a slightly higher percentage of response to *Meliphora* than have the controls, suggesting that in all probability the figures for *Meliphora* in Table VII err on the low side. As was pointed out earlier, the homogeneity of the series is not so satisfactory as the rest, and the error is probably associated with the high value of χ^2 as given at the foot of the table. But this slight discrepancy does not affect the final conclusion to be drawn from the class of observations, which is that "de-conditioning" does not entirely remove the effects of rearing on an abnormal host and that therefore a stimulus applied in the larval stage has its effect on the adult.

As is clearly shown by Tables V and VI, the experiments give no reason for supposing that the normal response to *Ephestia* is a conditioning effect. There seems no reason to doubt that it is germinally fixed. This view is confirmed by the experiments summarized in Table XII. In this case cocoons of *Nemeritis* reared on *Ephestia* were removed from their surroundings, cleaned as far as possible, and placed in gelatine capsules. It will be seen by comparing the results with Table III that there is no significant difference. Even though this process of isolating the cocoons is a very different matter to extracting the pupae themselves, yet it must undoubtedly reduce the intensity of odour to which the parasite is subjected and, were the response impressed anew on each generation and not germinally fixed, it would certainly produce some effect in behaviour.

Table XII—Responses to Larvae of *Ephestia* and *Meliphora* of eleven Individuals of *Nemeritis* (reared from *Ephestia*); Cocoons having been isolated in Gelatine Capsules

Exp.	*Ephestia*	*Meliphora*	Total
1	9	2	11
2	9	1	10
3	9	1	10
4	10	0	10
5	9	1	10
6	8	2	10
7	8	2	10
8	10	0	10
Total	72	9	81

= *Ephestia* 89 %; = *Meliphora* 11 %; standard error ± 5·5 %.

Table XIII—Comparison between Responses to *Ephestia* of *Nemeritis* reared from *Ephestia* and from *Meliphora*

	Ephestia	Blank	Total
Nemeritis ex *Ephestia*	*a* 186 = 87·3 %	*b* 27 = 12·7 %	213
Nemeritis ex *Meliphora*	*c* 298 = 84·5 %	*d* 55 = 15·5 %	353
Total	484	82	*N* 566

$$\chi^2 = \frac{(ad-bc)^2 N}{(a+b)(c+d)(a+c)(b+d)} = \frac{(10230-8040)^2 N}{213 \times 353 \times 484 \times 82} = \frac{2190^2 \times 566}{75200 \times 3962}$$

$$= \frac{4790000 \times 566}{29810000} = \frac{272000000}{29810000} = 9{\cdot}13, \quad \therefore P < 0{\cdot}01.$$

Finally the question arises whether there is any evidence for the inheritance of acquired characters revealed by this work. An examination of Table VI shows that the 8th generation shows a greater proportion, 39 %, in favour of *Meliphora* than does the first generation with 31·6 %. But an evaluation of χ^2 gives a result of only 1·78, therefore $P = 0{\cdot}20$ which implies that there is 1 chance in 5 that the result is an accidental one. Further, in studying Table VII it is seen that the 5th and 8th generations together show an actual decrease (not significant) in their preference for *Meliphora* as compared with the 1st generation; the figures being 61 % as against 68 % for the 1st generation. It is quite clear, therefore, that the work so far gives no indication of any inheritance of acquired characters.

Discussion

It is hardly possible to be too careful when using the results of such experiments as evidence bearing on problems of behaviour under the immensely complex conditions of the natural environment. It would be rash to suppose the behaviour of an insect in an air-conditioned olfactometer and the open air to be identical. But the demonstration of the possibility of "pre-imaginal olfactory conditioning" is certainly of peculiar interest in view of its possible importance in the early stages of evolutionary divergence.

As the senior author has previously pointed out (Thorpe 1930*b*) to postulate such evolutionary significance one does not need to invoke any Lamarckian theory. Any factor which will tend to split a population into groups will tend to aid the establishment of new variants in exactly the same way as do geographical barriers at a later stage in evolutionary divergence. It is true that, in the instance here described, rearing on the abnormal host does not, under olfactometer conditions with both hosts equally available, result in an actual preference for the new host over the old. But it does result in a marked decrease in the percentage of responses to the normal host, when both are offered. Now it must be realized that under natural conditions two hosts of a phytophagous or parasitic insect would hardly ever be equally available and were the conditions such that the alternative host became more readily available, then even a change of smaller magnitude than that described in the present work might well be sufficient to isolate a population effectively over a long period. During this period physiological or other modifications of genetical origin might arise and be conserved by the ecological barrier provided by the conditioned food preference, and so the establishment of a new race genetically distinct from the parent form could be produced.

Such in brief seems to be the main theoretical significance of olfactory conditioning. A further discussion of the subject will be found in the senior author's paper referred to above.

Finally it may be mentioned that rearing on *Meliphora* does not appear to produce any change in colour, size or structure of the adult parasite.

TABLE XIV—VALUE OF χ^2 AND P FOR DIFFERENT PAIRS OF RESULTS

Pairs of results Series	$\chi^2 = \frac{(ad-bc)^2 N}{(a+b)(c+d)(a+c)(b+d)}$	P
II and V	9·13	<0·01
III „ VI	25·30	<0·01
IV „ VII	15·30	<0·01
IV „ VIII	7·33	<0·01
III „ IX	0·24	0·6 approx.
III „ X	8·61	<0·01
IV „ XI	39·60	<0·01
VII „ VIII	1·151	0·28 approx.
VI „ X	11·66.	<0·01
1st and 8th generation ex *Meliphora*, choice *Ephestia*, *Meliphora*	1·78	0·20

The authors gratefully acknowledge their indebtedness to Dr. J. Wishart for his advice on the choice of statistical methods best suited to the type of data available.

SUMMARY

1. This paper describes experiments with the endoparasitic insect *Nemeritis canescens* (Grav.) (Hymenoptera, Ichneumonidae, Ophioninae), the normal host of which is the larva of the meal moth *Ephestia kühniella* (Zell.). Since *Nemeritis* is a parasite which finds its host almost entirely by the sense of smell which resides in the antennae, it was considered peculiarly suited to an investigation of the effect of the larval environment on the oviposition responses of the adult. The importance of the subject lies in its relation to the so-called "Hopkins Host-Selection Principle" and its bearing on the problem of the origin of biological races in insects and other animals. Although the genus *Ephestia* provides the only known hosts of this parasite in Europe, there exists in America a race which attacks the large wax moth, *Galleria mellonella* (Zell.).

2. It was found that although it is extremely difficult to rear the European race of *Nemeritis* on *Galleria* there is no great obstacle to securing oviposition on the larvae of the small wax moth *Meliphora grisella* (F.) provided that they were first placed in close contact with *Ephestia* larvae so as to be thoroughly contaminated with the smell of that host. Once the eggs are inserted in the body cavity of the *Meliphora* they develop normally and the larvae develop and adults are produced without any great mortality. No apparent changes in structure or colour result. The olfactory responses of *Nemeritis* reared in this way were compared with those of insects reared on the normal host by means of a McIndoo olfactometer of an improved type.

3. The experiments indicate that there is a strong germinally fixed tendency to follow up the odour of *Ephestia*. Insects which have been reared on *Ephestia* show no reaction whatever to the smell of *Meliphora*, but those reared from *Meliphora* while still preferring *Ephestia* show a significant attraction to *Meliphora* such that when given the alternative of the two hosts in the olfactometer the proportion choosing *Ephestia* is reduced from 85 to 65·8 %.

4. It is shown that some change in the olfactory responses may be brought about merely by exposing the *Nemeritis* to contact with the *Meliphora* larvae for a period immediately on emergence from the pupa. Further experiments indicate, however, that the whole of the effect of rearing on *Meliphora* is not due to this but that at least some conditioning effect results from the influence of the host acting during the pre-imaginal period.

5. It is shown that the odour of oatmeal, one of the usual food substances of *Ephestia* also exerts a slight attraction upon *Nemeritis*.

6. There is no evidence that continued rearing on the abnormal host over a number of generations increases the response to that host.

7. It is concluded that *Nemeritis* has an inherited oviposition response to *Ephestia* larvae, but that an additional oviposition response to the odour of *Meliphora*, a response which is entirely lacking in the normal insect, can be induced by contact with the new host. While this does not produce any diminution in the ability of the insect to respond to *Ephestia* when no other attraction is present, it does result in a significant reduction of the proportion which go to *Ephestia* when the two alternatives are equally available.

8. The application of these results to the problem of the origin of new races is discussed.

REFERENCES

Fisher, R. A. 1936 "Statistical Methods for Research Workers," 6th ed. Edinburgh and London: Oliver and Boyd.
Larson, A. O. 1927 *Ann. Ent. Soc. Amer.* **20**, 37–78.
McIndoo, N. E. 1926 *J. Econ. Ent.* **19**, 545–71.
Murr, L. 1930 *Z. vergl. Physiol.* **11**, 210–70.
Purewal, S. S. and Rao, P. K. 1936 *Misc. Bull. Imp. Counc. Agric. Res. Delhi*, No. 11, 1–37.
Quayle, H. J. 1926 *Bull. Calif. Agric. Exp. Sta.* No. 402, pp. 33.
Richmond, R. G. 1925 *J. Econ. Ent.* **18**, 425.
Salt, G. 1935 *Proc. Roy. Soc.* B, **117**, 413–35.
von Stein-Beling, I. 1934 *Biol. Zbl.* **54**, 147–69.
Thompson, W. R. and Parker, H. L. 1928 *Bull. Ent. Res.* **18**, 359–64.
Thorpe, W. H. 1929 *J. Linn. Soc. Zool.* **36**, 621–34.
— 1930*a* *J. Linn. Soc. Zool.* **37**, 489–92.
— 1930*b* *Biol. Rev.* **5**, 177–212.
Ullyett, G. C. 1936 *Proc. Roy. Soc.* B, **120**, 253–91.

612.843.61

The Luminous Efficiency of Light entering the Eye Pupil at Different Points and its Relation to Brightness Threshold Measurements

BY B. H. CRAWFORD

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I—INTRODUCTION

In previous papers (Crawford 1936) results have been given for the variation of pupil size and of brightness threshold (measured with small artificial pupil) with time after changing from a high to a low general light stimulus. These results cannot, however, be immediately combined to give the response of the eye as a whole (with natural pupil) until the possible effects of the efficiency of light entering the eye through different parts of the pupil have been investigated. It has been found (Stiles and Crawford 1933) that light entering the eye near the periphery of the pupil is less efficient in producing the sensation of brightness than light entering near the centre. Probably the same thing occurs for the threshold of the sensation of brightness, so that the threshold would be higher for light passing through the more peripheral parts of the pupil. The first part of this paper is concerned with the testing of this hypothesis. The results are expressed in the form of curves connecting brightness threshold with distance of the point of entry of the light from the centre of the pupil, these curves being called, for convenience, threshold efficiency curves.

The measurements of this effect made hitherto have related to the foveal region of the retina and a fairly high field brightness. They are here extended, by the threshold brightness method, to regions of the retina more or less distant from the fovea and to field brightnesses extending down to zero. In order to complete the picture of the luminous efficiency effect, in so far as it relates to white light, it remains only to investigate the course of the variation in shape of the threshold efficiency curve with time after the field brightness is changed suddenly from one value to another. One case only has been dealt with, namely, the change from a high value of field brightness to zero. Finally, all these results may be combined with the corresponding measurements of pupil size and retinal sensitivity to give the response of the complete eye.

2—APPARATUS AND METHODS OF MEASUREMENT

The apparatus is arranged to give in the field of view of the subject a uniformly illuminated area on which can be superposed a test spot and the necessary fixation points. The general plan is shown in fig. 1. All parts of the field are seen by Maxwellian view. The main beam providing a general illumination of the field is fixed, and a sealing-wax mouth grip locating the subject's head and eye can be moved on slides until the beam enters through the centre of the eye pupil. The test-spot beam can be traversed horizontally across the pupil by a calibrated micrometer screw which rotates slightly the mirror Q; the cross-section of this beam where it passes the eye pupil is approximately $0{\cdot}8 \times 0{\cdot}3$ mm. The point of entry of the fixation-point beam is unimportant, and coarse adjustment only is provided for this. The intensities of the main and test-spot beams are controlled by neutral wedges of fairly shallow gradient, with neutral filters for large steps of intensity. The intensity of the fixation-point beam is regulated by varying the current through the lamp. Shutters are provided for exposing the main field and the test spot independently for any desired periods. The wedge in the test-spot beam is attached to a carriage provided with electrically operated pens by which the subject of experiment can record, for a given wedge setting, whether the test spot is visible or in-visible. By this means readings can be taken by the multiple decision method (Crawford 1937), which has been found to give the most accurate and unbiased results in the shortest time.

As in previous papers, the positions of the various components of the field of view are specified in degrees subtended at the eye, positions and directions in the external field being referred to in all cases, not retinal positions. Brightness measurements were made by comparing a small part of the Maxwellian field with an illuminated white diffusing screen, with an artificial pupil at the eye. The diameter of the latter was 2·96 mm. The brightness of the diffusing screen was adjusted to appear equal to that of the Maxwellian field and was then measured with an illuminometer. In all the measurements described here the test field was round, of diameter 0·46°, exposed for 0·05 sec. All fields were white, of colour temperature approximately equal to that of a vacuum electric lamp. The filters used were not all perfectly neutral, however, giving slight irregular variations in field colour, though none were large enough to be noticeable without a standard of comparison.

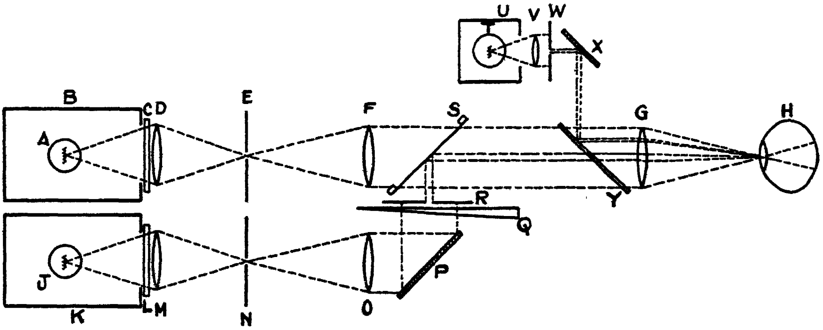

FIG. 1—Plan of apparatus. *A*, lamp for general field illumination; *B*, light-tight box; *C*, neutral filter; *D*, lens forming image of *A* at *E*, aperture and shutter; *F*, lens rendering beam of light parallel; *G*, lens converging beam to a focus in plane of pupil of *H*, the subject's eye; *J*, lamp for test field illumination; *K*, light-tight box; *L*, neutral filter; *M*, lens forming image of *J* at *N*, aperture and shutter; *O*, lens rendering beam of light parallel; *P*, mirror; *Q*, neutral wedge; *R*, aperture limiting test-field size; *S*, pellicule mirror (thin film of celluloid); *T*, lamp for fixation point illumination; *U*, light-tight box; *V*, lens giving parallel beam of light; *W*, aperture; *X*, mirror; *Y*, plane glass mirror.

The procedure adopted for the determination of threshold efficiency curves has been designed to eliminate, so far as possible, any effects of fatigue of the retina. A number of suitably spaced points of entry through the pupil are chosen, one being at the centre, and the test-spot beam is adjusted to pass through each in turn, starting at the point nearest to the nasal edge of the pupil and passing across to the other edge. A determination of brightness threshold is made at each point and the points are then taken in reverse order. Any effects of fatigue are at once evident, since the first and last threshold readings are taken at the same point. In actual practice, fatigue, in the sense of rise in threshold brightness, is very rarely found, and it has been assumed that it is sufficiently eliminated by using the mean values of the threshold readings taken at each point of entry.

3—COMPARISON OF LUMINOUS EFFICIENCY MEASUREMENTS MADE BY THE PHOTOMETRIC MATCHING AND THE THRESHOLD METHODS

A threshold efficiency curve determined for subject B. H. C. by the method outlined above is shown in fig. 2 (circles). The general field brightness of 22 c./ft.^{2} remained constant throughout the experiment, so that the change in threshold may be interpreted, as in the photometric matching

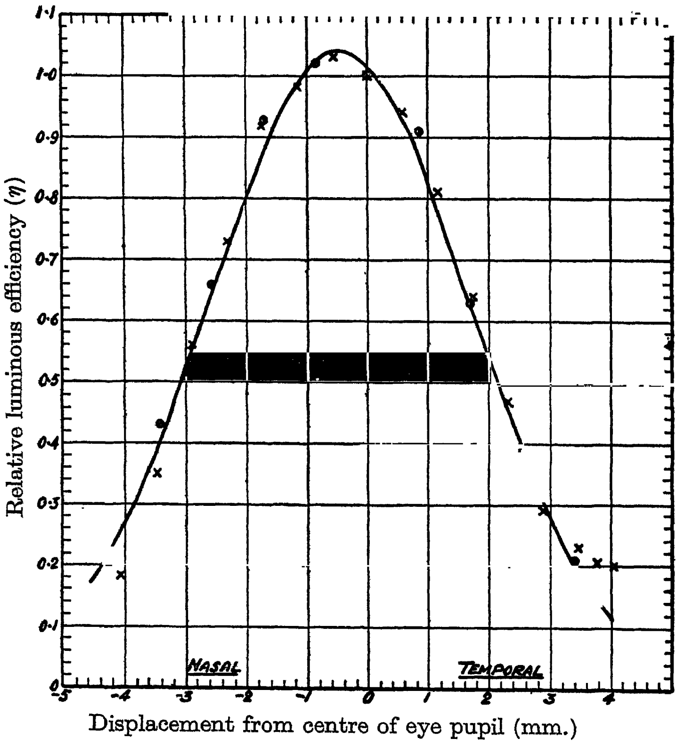

FIG. 2—Comparison of efficiency measurements by photometric matching and brightness threshold methods. ×, measurements by photometric method; ⊙, measurements by brightness threshold method; curve, $\eta = 1{\cdot}04e^{-0{\cdot}108(r+0{\cdot}47)^2}$.

method, as a change in the efficiency of the eye in receiving light along various paths. On the same diagram are shown earlier results for the same subject by the photometric matching method (crosses) taken from the paper by Stiles and Crawford 1933. The relative efficiencies are arbitrarily equated to unity at the centre of the pupil. The agreement between the results obtained by the two methods is almost perfect, and such small deviations as there are lie well within the limits of experimental error. The curve drawn through the points has the formula

$$y = 1{\cdot}025e^{-0{\cdot}105(r+0{\cdot}47)^2}.$$

It is interesting to note that it fits the points well over a wide range and lends support to one of the suggestions put forward by Wright and Nelson (1936) to explain the origin of this variation in luminous efficiency, namely, that light must pass down the axis of a cone in order to stimulate it, and that the orientation of the cone axes follows some law of random distribution about a mean value. On these suppositions, the luminous efficiency curve would represent the law of random distribution, and it is, perhaps, significant that the curve actually found is the commonly occurring Gaussian probability curve.

Apart from possible theoretical consequences, however, the formula is convenient for purposes of calculation. A formula of the above type has been found to represent all the results so far obtained with good enough approximation for most practical purposes.

4—DEPENDENCE OF EFFICIENCY ON RETINAL TEST AREA AND ON FIELD BRIGHTNESS

Threshold efficiency curves have been determined for parts of the retina other than the fovea and at various field brightnesses down to zero. In the latter condition no stimulus is applied to the eye except the test spot and a dim fixation spot, and the condition of the eye can therefore approach closely to that of complete dark adaptation. Figs. 3 and 4 show results obtained by two subjects for a number of retinal areas at field brightnesses of 22 c./ft.^{2} (broken line curves) and zero (continuous line curves). The logarithms of the threshold efficiencies have been plotted on these graphs so that the shapes of the different curves can be compared directly without any elaborate adjustments of scale. Each curve has been given the arbitrary value of $\log_{10}\eta = 0$ at the pupil centre. For both subjects the foveal curves are practically the same at the two brightness levels. Such

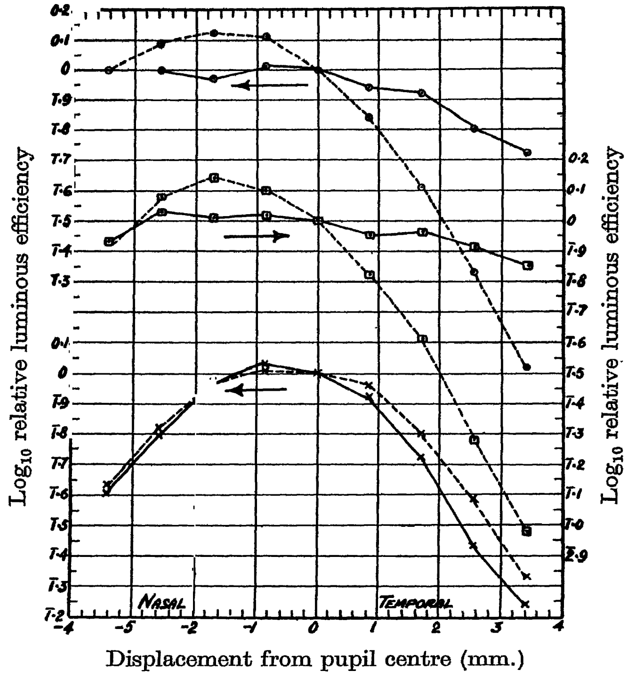

Fig. 3—Polar efficiency curves at high and zero field brightnesses for different retinal areas. Subject B. H .C. Continuous curves, zero field brightness; dotted curves, field brightness = 22 c./ft.². ×, test area at fovea; ⊡, test area 5° from fovea; ⊙, test area 14° from fovea.

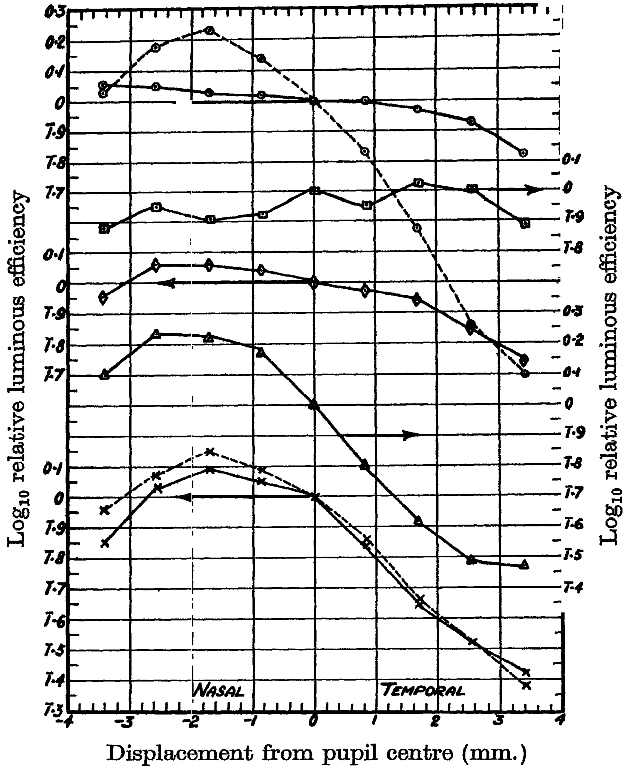

Fig. 4—Polar efficiency curves at high and zero field brightnesses for different retinal areas. Subject H. R. S. Continuous curves, zero field brightness; dotted curves, field brightness = 22 c./ft.². ×, test area at fovea; △, test area ½° from fovea; ◇, test area 1° from fovea; ⊡, test area 2° from fovea; ⊙, test area 5° from fovea.

difference as there is hardly exceeds the probable error of the measurements. At the parafoveal test areas matters are very different. Here, both subjects show a marked difference between curves at high and zero field brightnesses: those at the high brightness approximate in shape to the foveal curves; those at zero brightness have almost, though not quite, flattened out, so that the luminous efficiency of a light pencil becomes almost independent of its point of entry through the eye pupil. The results for subject H. R. S. include a number of retinal test areas located at comparatively short distances from the fixation point. These demonstrate clearly the existence of a rather sharply defined foveal area, outside which the efficiency curve assumes at once a typical parafoveal shape.

These results are of considerable interest in relation to theories of the origin of the efficiency effect. Whatever mechanism is postulated as giving rise to the variation of efficiency with angle of incidence on the retina, must also allow for variation with field brightness. There are two main possibilities, a mechanical movement of some structure in the retina or a photochemical change. The latter alternative is not impossible; it would require a phototropic substance in the retina. The experimental facts are not, however, in very good accord with the phenomena exhibited by phototropic substances. These, in the dark state, can absorb light of a certain wave band and become thereby transformed so that they absorb light of some longer wave band. In the case of the postulated retinal pigment, on the other hand, white light is incident on it in its initial transparent state and must transform it so that it absorbs uniformly over the visible spectrum, which is not the sort of thing observed with phototropic substances. However, experiments with coloured fields would be necessary to decide definitely one way or the other. An additional objection to the phototropic substance hypothesis is that a non-phototropic pigment would be required at the fovea. The mechanical movement of some portion of the retinal structure is, perhaps, more promising. Assuming that the falling off in luminous efficiency with increasing angle of incidence on the retina is due to embedding of the recipient elements, rods or cones, in a more or less opaque pigment, the increase in efficiency in the dark might be due to migration of the pigment away from the recipient elements, or to pulling out of the recipient elements from the pigment layer. Both these mechanisms have been demonstrated in various animals, mostly of a low order, but not with certainty in man. Either would lead qualitatively to the experimental results described above.

The manner in which the shape of the parafoveal efficiency curve changes when the field brightness varies from a high value to zero is shown, for two subjects, in figs. 5 and 6. The parafoveal retinal area was taken at 5° from the fixation point (fovea centralis). For both subjects the curves show a progressive flattening as the field brightness falls, without any definite indication of change in position of the maximum. If the curves are fitted with a formula of the type mentioned above,

$$y = ae^{-b(r+c)^2},$$

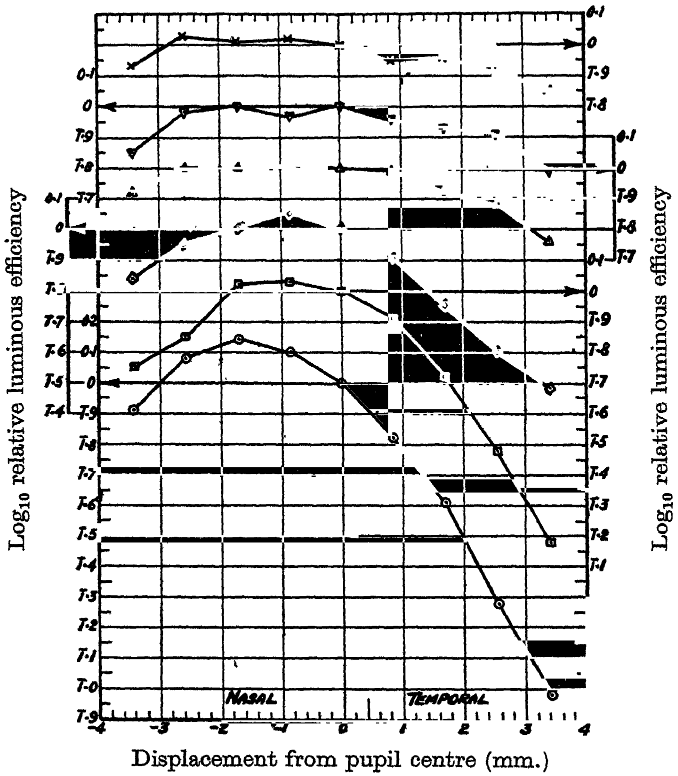

FIG. 5—Polar efficiency curves at various field brightnesses (B) for a retinal area 5° from the fovea. Subject B. H. C. ×, $B=0$; ▽, $B=0\cdot000060$ c./ft.^{2}; △, $B=0\cdot00107$ c./ft.^{2}; ◇, $B=0\cdot0102$ c./ft.^{2}; ⊡, $B=0\cdot096$ c./ft.^{2}; ⊙, $B=22$ c./ft.^{2}.

the change in shape is shown by a decrease in the value of b with decrease in field brightness. The value of b for any given set of experimental points is most accurately determined by finding the curve which best fits the points, but a good approximation may be made by taking the logarithm of the ratio of any two points; if the above formula actually fitted the experimental points this would be proportional to b.

$$\log_{10}\eta = \log_{10}a - b(r+c)^2 \log_{10}e;$$

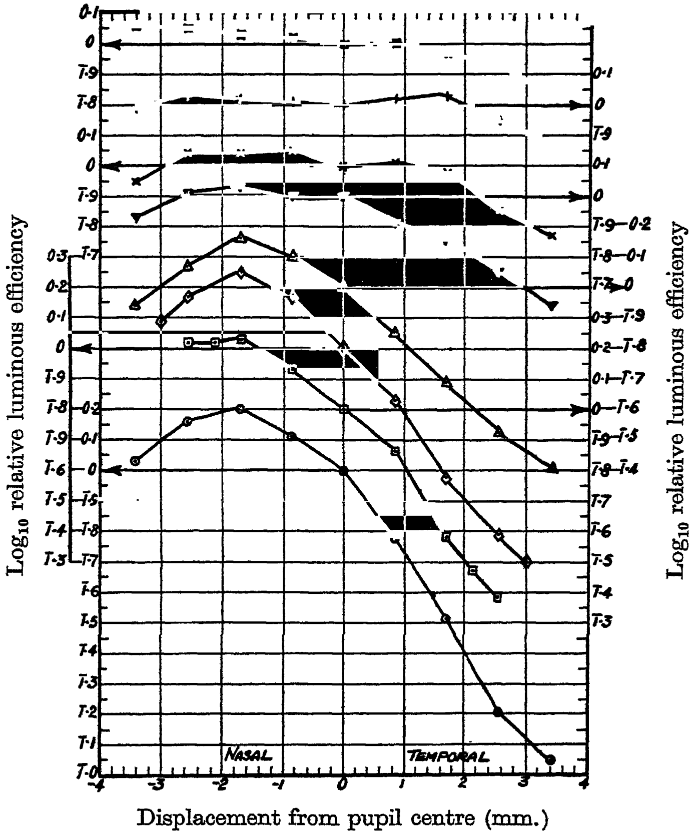

FIG. 6—Polar efficiency curves at various field brightnesses (B) for a retinal area 5° from the fovea. Subject H. R. S. ⊞, $B=0$; +, $B=0{\cdot}000060$ c./ft.^{2}; ×, $B=0{\cdot}00107$ c./ft.^{2}; ▼, $B=0{\cdot}0102$ c./ft.^{2}; ▲, $B=0{\cdot}096$ c./ft.^{2}; ◇, $B=1{\cdot}29$ c./ft.^{2}; ⊡, $B=8{\cdot}9$ c./ft.^{2}; ⊙, $B=22$ c./ft.^{2}.

so that,

$$\log_{10}\eta_1-\log_{10}\eta_2=\log_{10}\frac{\eta_1}{\eta_2}=-b\{(r_1+c)^2-(r_2+c)^2\}\log_{10}e$$
$$=b\times\text{constant}.$$

This gives a convenient method for following the course of the change in shape of the efficiency curve, namely, plotting b values deduced from the ratio of efficiencies at two points on the curve against the variable, such as field brightness, concerned. For the sake of accuracy, one point should be near the peak of the efficiency curve, the other some distance from it. Such curves are shown in fig. 7, curves 1 and 2, for the two subjects. For H.R.S. the points are deduced from the data of fig. 6. For B. H. C. there are, in addition to points taken from fig. 5, points which were determined by a direct measurement of the ratio of efficiencies at two entry points using a series of field brightnesses. Fig. 7 shows clearly that there is a large range of low field brightnesses over which the shape of the efficiency curve remains approximately constant and of the flattened form characteristic of zero field brightness. The b value then starts to rise, more or less abruptly, at a brightness of about 0·001 c./ft.^{2} and tends to asymptote to a maximum value at about 1 c./ft.^{2}. It may be noted that the field brightness at which the shape of the efficiency curve begins to change is also that at which the parafoveal brightness threshold rises above the

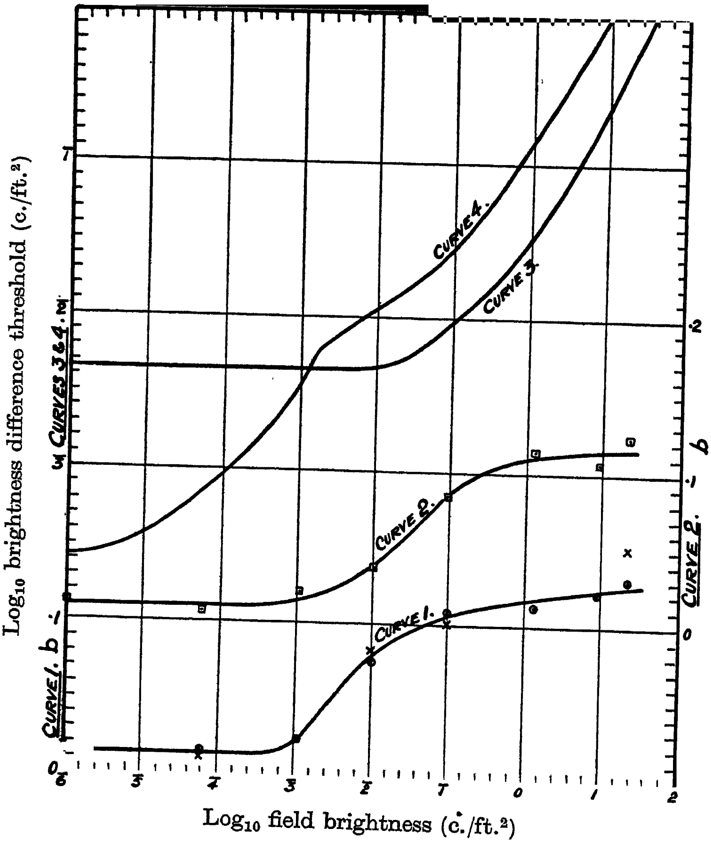

FIG. 7—Curve 1, variation with field brightness of constant b in formula

$$\eta = ae^{-b(r+c)^2}$$

fitted to polar efficiency curves for subject B. H. C. Curve 2, same for subject H. R. S. Curves 3 and 4, variation of brightness threshold with field brightness for foveal and parafoveal (5°) vision respectively for subject H. R. S.

foveal value and there is a kink or inflexion in the parafoveal threshold curve (see curves 3 and 4, fig. 7). This more or less sudden change in shape of the parafoveal threshold curve has been interpreted as due to a change-over from one mechanism of vision to another, from twilight to daylight vision, and it is of great interest that the change-over is apparently closely associated with the commencement of pigment migration or whatever else it may be that gives rise to this change in luminous efficiency. It would seem that the pigment migration is called into play by stimulation of the daylight mechanism of vision, but is unconnected with the twilight mechanism; as the daylight mechanism is increasingly stimulated the pigment migrates to a greater extent between the recipient elements until it reaches the limit of its motion.

5—TIME VARIATION IN SHAPE OF EFFICIENCY CURVE AFTER SUDDEN CHANGE IN GENERAL FIELD BRIGHTNESS

It has been established that the parafoveal efficiency curve is much flatter at zero brightness than at a high brightness. It remains, therefore, to investigate the time course of the change in shape of the efficiency curve when a high brightness is suddenly cut off. The case of a change from low to high brightness has not yet been considered, while cases of change from a high to a lower brightness can be deduced from the high to zero brightness case. The measurement of variation in shape of the efficiency curve cannot be done by any very direct method; the only way is to choose a number of entry points through the pupil and determine at each the curve connecting brightness threshold with time after cutting off the initial field brightness. These measurements are made as described by Crawford (1936). The shape of the efficiency curve at any required time can then be deduced from the threshold readings at that time. The complete procedure carried out in this way would be extremely tedious and the method has been used in an abbreviated form. It has been assumed that the efficiency curve remains throughout of the same exponential type, altering only in the value of b. It is then sufficient, in order to determine the course of the change in shape of the efficiency curve, to take two pupil entry points, one near the peak of the curve, the other some way from it, and determine at each a curve of brightness threshold against time. The value of b may then be deduced for any time after the start.

Fig. 8 shows the results obtained for two subjects. In both cases the initial field brightness was 22 c./ft.^{2} and the retinal area tested was 0·46°

in diameter at 5° from the fixation point. The individual curves of brightness threshold against time are shown in curves 1 and 2 for subject B.H.C., in curves 4 and 5 for subject H.R.S., curves 1 and 4 being for an entry

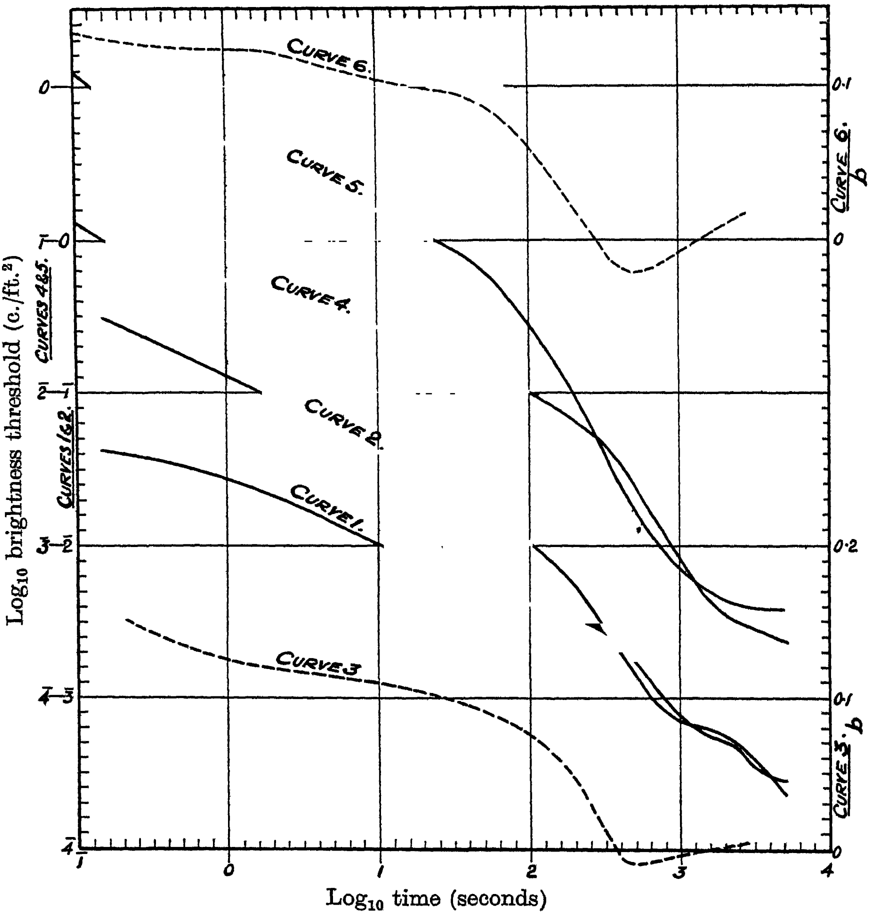

FIG. 8—Curve 1, variation of brightness threshold with time after cutting off initial field brightness of 22 c./ft.^{2} for test beam entering eye pupil 0·85 mm. to nasal side of centre; curve 2, same for test beam entering eye pupil 2·56 mm. to temporal side of centre; curve 3, b value deduced from difference between curves 1 and 2: subject B. H. C., test area 5° from fovea. Curves 4, 5, 6 same as above for subject H. R. S.

point near the maximum of the efficiency curve, 2 and 5 as far away from the maximum as the pupil size would allow. From the vertical separation of these curves has been calculated a series of values of b for the two subjects, assuming that for subject B. H. C. the maximum of the efficiency curve is 1·0 mm. to the nasal side of the pupil centre, for subject H.R.S. 1·6 mm. to the nasal side of the pupil centre. These assumptions are derived from the results already described for the efficiency curve under static conditions. The results are very similar in the two cases, though no definite significance can be attached to the negative values of b, even though shown by both subjects, owing to the relatively low accuracy of the threshold measurements. Apart from this, however, it would seem that the mechanism of the efficiency change is the same for the two subjects tested. Most of the change in b takes place between 1 and 5 min. from the start, that is, in about 240 sec. On the pigment migration hypothesis, the distance through which the pigment must move in this time is the length of a rod or cone, which leads to an approximate average speed of migration of 0·0002 mm./sec. Such a speed does not appear to be inconsistent with a mechanism of the pigment migration or rod retraction type. If both occurred together the speed of each would be halved. The rate appears to be more rapid, however, than for pigment migration in the frog's eye, where the complete process is said to take 1–2 hr. after cutting off a high illumination (Arey 1916). This may possibly explain why the phenomenon has not been definitely demonstrated in man: by the time the eye has been excised and fixed for examination the pigment may well have migrated back to its dark location. It is possible that this difference in rate of pigment migration in frog and man may be due largely to difference in body temperature, since Bennitt (1924) found the migration effect in the eyes of various crustaceans to have a definite and fairly large temperature coefficient.

6—APPLICATION OF RESULTS TO TIME COURSE OF ADAPTATION WITH NATURAL PUPIL

The results given in previous papers (Crawford 1936, 1937) for the time change of brightness threshold after cutting off an initial conditioning stimulus and for the time change of pupil diameter under similar circumstances can now be compounded with the data of the previous section to give the time change of brightness threshold including the effect of the natural pupil of the eye; the results mentioned above relate to brightness threshold measured with a small, fixed, artificial pupil. The computation is as follows. At any given time after the cut-off of the initial conditioning stimulus the pupil size is known, also the shape of the threshold efficiency curve as defined by its b value. The *effective* pupil area can be calculated from these data; if the efficiency curve and pupil opening are not concentric, as is usually the case, the necessary integration has to be done graphically. The brightness threshold measured with the artificial pupil is then multiplied by the ratio of the effective areas of the natural and artificial pupils; the effective area of the artificial pupil may be taken as the actual area if it is small. The result should tally with that obtained by direct measurement with the natural pupil. In order to test this, curves showing variation of brightness threshold with time were determined for the two subjects of the previous experiments, using both artificial and natural pupils. The various runs were intermingled with each other and taken within as short a space of time as possible so as to minimize any effects due to sporadic changes of threshold. The results for the two subjects are shown in fig. 9. In each set, the continuous curves are determined by direct measurement, the upper one relating to the artificial pupil, the lower to the natural pupil, while the dotted curve is obtained by calculation from the artificial pupil curve and the data of the present paper. The dotted curve should agree with the lower continuous curve in each set. The agreement found is fair, but not striking. On the whole, the discrepancies are not greater than the possible errors of measurement, especially when it is considered that the various data which made up the calculated curves were obtained at periods separated by several months. The pupil size factor is probably the most important as a source of error. The small consistent error shown at the ends of the curves might possibly be due to erroneous assumptions regarding the shape of the efficiency curve. Inevitably, the experimental determination of the latter cannot extend to the extreme edges of the pupil opening, and it is possible that a rapid drop in efficiency exists here which would effectively narrow the pupil opening sufficiently to explain the discrepancy shown. On the other hand, there is little reason to suppose that the discrepancy is not due to the ordinary chance errors of these measurements, even though it is shown by both subjects. The present results therefore support the method of combining a number of visual factors which have been determined separately.

Grateful acknowledgement is here made of the assistance given in this work by Mr. H. R. Sayer. The work was carried out under the auspices of the Illumination Research Committee of the Department of Scientific and Industrial Research.

SUMMARY

The luminous efficiency of light entering the eye through different parts of the pupillary opening has been measured by determination of the

threshold brightness corresponding to the various light paths, and compared with previous results (Stiles and Crawford 1933) obtained by a

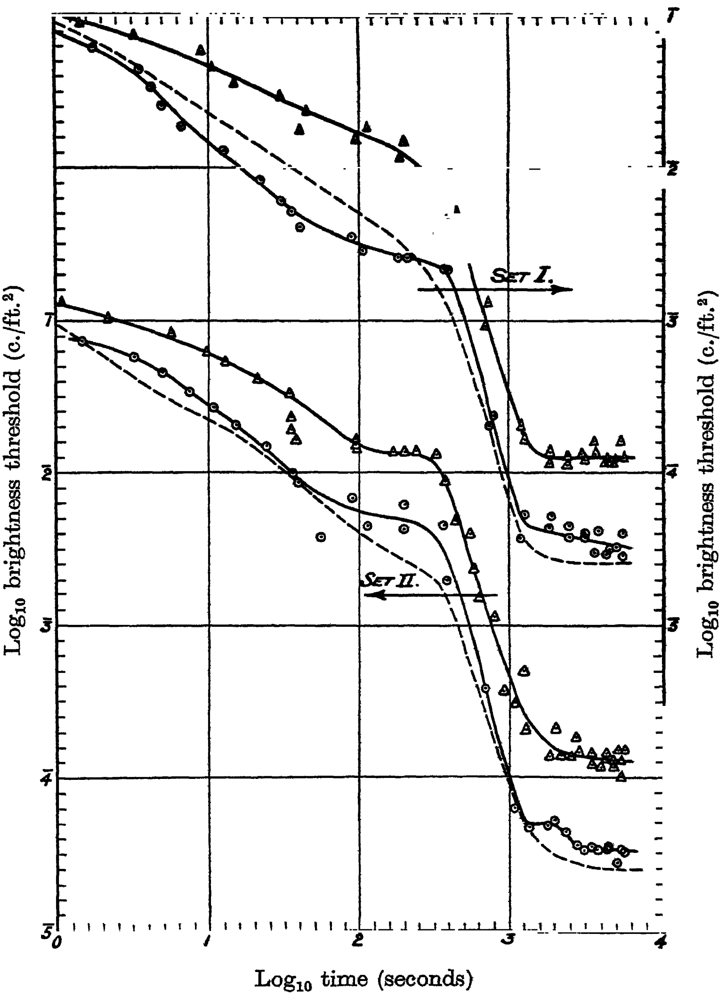

FIG. 9—Variation of brightness threshold with time after cutting off initial field brightness of 69 c./ft.^{2} with natural and artificial pupils. △, artificial pupil; ⊙, natural pupil; dotted curve, natural pupil calculated from artificial pupil and data for variation of pupil size and efficiency. Set I, subject H. R. S., retinal area 5° from fovea; set II, subject B. H. C., retinal area 14° from fovea.

method involving photometric matching of brightness. The results by the two methods are in close agreement, pointing to the probability that the same visual mechanism is employed in both types of measurement.

The threshold method has been applied to the investigation of luminous efficiency for various regions of the retina at a series of levels of brightness of the field of view from zero up to 22 c./ft.^{2}. For parafoveal retinal areas the efficiency varies with brightness, exhibiting a well-defined maximum near the centre of the pupil at high brightnesses, but becoming almost constant over the area of the pupil at low and zero brightnesses. This is in contrast to the foveal area, for which the efficiency distribution over the pupillary opening is practically unaffected by the field brightness, having always a maximum near the centre of the pupil.

The threshold method has been further applied to the determination, for parafoveal retinal areas, of the rate of change of efficiency distribution after a high brightness has been changed suddenly to zero. The rate does not appear to be inconsistent with the hypothesis of pigment migration or other mechanical movement of the retinal elements put forward to explain the phenomena observed.

These results have been combined with other measurements (of pupil size and of variation of threshold brightness using an artificial pupil) to give the response of the eye with its natural pupil, and compared with measurements made directly with the natural pupil. Agreement is fair and supports the method of combining a number of visual factors which have been determined separately.

REFERENCES

Arey 1916 *J. Comp. Neurol.* **26**, 121, 213.
Bennitt 1924 *J. Exp. Zool.* **40**, 381.
Crawford 1936 *Proc. Roy. Soc.* B, **121**, 376.
Crawford 1937 *Proc. Roy. Soc.* B, **123**, 69.
Stiles and Crawford 1933 *Proc. Roy. Soc.* B, **112**, 428.
Wright and Nelson 1936 *Proc. Phys. Soc.* **48**, 401.

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The Experimental Determination of the Time of Chromosome Doubling

By K. Mather

Galton Laboratory, University College, London

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The problem of the stage of the mitotic cycle at which the chromosomes split, "divide", or become double, is one that has inevitably received much attention of recent time, bearing, as it does, on the general theory of chromosome behaviour. There has developed a greater diversity of opinion on this subject than on any question of the external relationships of the chromosomes.

It is generally considered that there are two schools of thought on this question: (*a*) those who consider that the chromosomes split during the resting stage immediately prior to the mitosis at which their halves separate, and (*b*) those who consider that the chromosomes split at the previous mitotic metaphase, or even earlier. The ostensible uniformity of the latter opinion is fictitious, since this group includes those maintaining that the split occurs at almost all the different stages of the mitotic cycle previous to that at which separation occurs, or, in some cases, even at stages of various previous cycles. It is superfluous to give detailed lists of the various hypotheses advanced, as Darlington (1935, 1936), Gustafsson (1936) and Lorbeer (1934) have dealt with the various shades of opinion in a sufficiently thorough manner.

The evidence on which the great majority of the contending views are based is mainly observational, i.e. visual observation of the chromosomes using light of the visible spectrum, and photographic observation using various types of radiation just outside the visible range. The structures described are usually of a magnitude similar to the wave-length of the radiation used and situated at distances from each other below the definition of the microscope. These observations could not be considered as critical or final, even if they were concerned with uncoagulated systems and gave consistent results. If further evidence of this were needed it is provided by the very contradictions which the observations of various authors show. Some approach giving more consistent results is necessary.

Mather and Stone (1933) pointed out that the effect of X-radiation in breaking the chromosomes could be used for this purpose. This possibility appears to have been generally agreed upon. The assumption essential to analysis by this means is that if the chromosome is double at the time of breakage only one of its two chromatids will be affected. Chromosomes which divide after breakage will, on the other hand, show ruptures of both chromatids at the same level. Then by timing the fixations to come at certain intervals after treatment, mitotic metaphases showing the effect of irradiation at various stages of the cycle will be obtained. Thus an easily detectable difference, the symmetry or otherwise of the metaphase chromatids, which are large structures, is used to detect a difference that is not itself capable of exact observation by direct methods. An attempt to apply this method to irradiated root tips of *Crocus* sp. gave results strongly indicating a split during the late resting stage immediately prior to the mitosis concerned. These observations have been criticized on the ground that it was not certain that the mitoses observed were those immediately following the irradiated resting stage.

An essential refinement introduced by Huskins and Hunter (1935) removes the basis of this criticism. They used the first mitotic divisions of pollen grains for their observations. The interval between meiosis and pollen-grain mitosis is sufficiently great to allow of certainty of irradiation during the intervening resting stage. These authors obtained results with *Trillium* which, they claim, contradict those of Mather and Stone, in that chromatid breaks, i.e. breaks affecting but one of the two chromatids at a certain level, were obtained. They omitted, however, one of the essential features of the technique as devised by Mather and Stone, viz. the use of a number of fixations at different times after irradiation. As will be seen below, this omission vastly increased the difficulty of interpretation of their results.

White (1935) irradiated spermatogonial tissues of the locust and obtained chromosome breaks in fixations made both before and after others in which chromatid breaks were found. These results he considered to show the problem to be of great complexity and probably incapable of solution by these means. A possible explanation of his results is given by Darlington (1936), and no one else has reported similar findings. The possible objections to spermatogonial observations do not apply to pollen grains, where successive divisions can be distinguished.

A superficially similar experiment has been performed and reported by Nebel (1936). He irradiated meiosis in *Tradescantia* and obtained a small number of what he considers to be chromatids broken across *half* their width at second anaphase. This attempt to observe half-chromatid breakage entirely defeats the object of the X-ray method as generally applied. It is simply replacing difficult observation on untreated material by similar observation of the same structure after a form of treatment. The crucial test of Nebel's case would be given by observations on the pollen-grain divisions. If his half-chromatid breaks were real they should show as *chromatid* breaks at pollen-grain division. The frequent occurrence of such configurations would leave no doubt about the matter. Realizing this, Nebel cites Mather (1934) as having illustrated such breaks. The figures of this latter paper seem to show nothing which could legitimately be interpreted as chromatid breaks at pollen-grain division. There are very small pieces of chromosome which, as we should expect, fail to attain the normal width of the chromosomes before becoming spherical, but all the chromosomes of sufficient size to reach an elongated shape are shown as double and symmetrical. The half-chromatid breaks cannot therefore be considered as demonstrated.

In view of the equivocal nature of evidence outlined above, it was decided to perform experiments using Mather and Stone's technique incorporating Huskins and Hunter's improvement. Similar experiments have been performed and reported independently by Riley (1936). His results will be considered in conjunction with the present ones.

I am indebted to Dr. A. C. Fabergé for invaluable assistance during the experiments.

MATERIAL AND METHODS

Three diploid species of monocotyledonous plants were chosen as experimental material, viz. *Tradescantia bracteata*, *Eremurus Tubergeni* and *Allium Moly*. This choice was made to ensure that, in each case, material was from the same plant or clone (in *Allium* this was not known with complete certainty), that the chromosome number was reasonably low, and that a sufficiency of pollen-grain mitoses was obtainable.

The material was irradiated by a Coolidge water-cooled tube with a tungsten anticathode using no filter. The dosage was administered as follows:

Tradescantia 70 V, 5 mA, 30 cm. distance for 15 min. (about 650 r. units).
Eremurus and *Allium* as for *Tradescantia* but for 7½ min.

Fixations were made at intervals after treatment as shown in Table I.

The smear technique was employed and the fixative was 2 BE. Staining was done by the gentian violet-iodine method.

Table I

	Time of fixation after treatment hr.	Type of breaks
Tradescantia	24	Chromatid
	36	Mainly chromatid
	48	Mixed
	60	Mainly chromosome
	72	Chromosome
Eremurus	32	Chromatid
	56	Chromatid
	78	Chromosome
	103	Chromosome
Allium	48	Chromatid
	60	Chromatid
	72	Chromatid
	160	Chromatid
	192	Very few divisions observed
	214	Chromosome

RESULTS

The first experiment, that using *Tradescantia*, gave the clearest results of which a summary will be found in Table I. The very first fixation, made at 12 hr. after treatment, gave no analysable divisions and is omitted from the table. The 24 hr. material showed nothing but chromatid breaks, such as chromatid fragments and chromatid interchanges (White 1935; Huskins and Hunter 1935). These were found in great profusion. At 36 hr. after treatment chromosome breaks were found in a few nuclei, but the vast majority were showing chromatid alterations. Fixations made 48 hr. after treatment still showed some chromatid breaks, but rather more nuclei showed chromosome rearrangements. In the later fixations practically no chromatid breaks were observed but chromosome changes were common. These were of the types described by Mather and Stone (1933). Chromatid breaks were an extreme rarity in the 72 hr. material. This was confirmed from other material fixed for a different purpose at 72 hr. after irradiation. These chromatid breaks were always found one in a nucleus, side by side with chromosome breaks. This is in definite contrast to the material observed 36 hr. after irradiation where both types of break were found but rarely, if ever, in the same nucleus. This contrast of behaviour would appear to imply contrasting causes of the chromatid breaks in the two fixations. The chromatid breaks in late fixations could be due either to delayed fusion or damage preventing perfect chromosome reproduction. Where chromatid breaks were common no case was observed of both chromatids being broken at the same level or even at approximately the same level.

These results and those of Riley (1936), who used similar material, are in complete agreement.

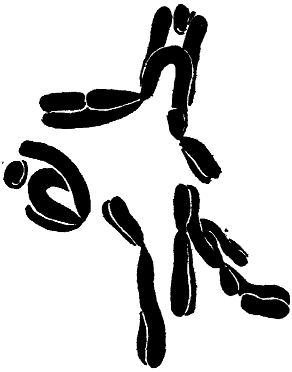

FIG. 1

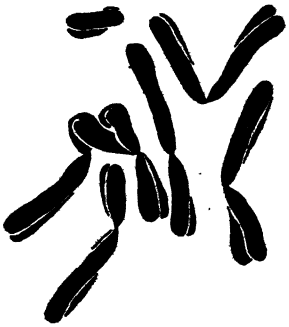

FIG. 2

FIG. 1—Pollen-grain mitosis in *Tradescantia*, 24 hr. after treatment, to show typical chromatid breaks and rejoins. The chromosomes are somewhat spaced out for clarity. × 3000.

FIG. 2—Pollen-grain mitosis in *Tradescantia*, 72 hr. after treatment, to show typical chromosome breaks and rejoins. The chromosomes are somewhat spaced out for clarity. Compare with fig. 1. × 3000.

The observations on *Eremurus* and *Allium* are not so extensive owing to technical difficulties, but it is clear that they confirm the main features of the *Tradescantia* scheme. The earlier fixations always showed chromatid breaks, and the later ones chromosome breaks. The number of divisions examined in *Eremurus* was relatively small.

The precise time at which the change from chromatid to chromosome breaks occurred differed, however, in the different plants. In *Tradescantia* it was at roughly 36–48 hr., in *Eremurus* 56–78 hr., and in *Allium* chromosome breaks were not found until fixations made 214 hr. after treatment were examined.

In this last case it was thought advisable to make observations to confirm that treatment had indeed been applied during the post-meiotic resting stage, and not during meiosis itself. These confirmatory observations were of two kinds. First, buds were carefully opened and one anther removed to determine the stage of cytological development. The bud was then closed and labelled. It was opened again after a suitable size had been attained and the stage of development again determined. Thus it was possible to ascertain the time which had elapsed between the second meiotic division and the pollen-grain division. Second, it was found that buds irradiated during meiosis always gave pollen grains possessing micro-nuclei (see also Mather 1934). Hence the pollen grains not containing micro-nuclei were irradiated after the end of meiosis. It appeared that pollen-grain mitosis occurred about 240 hr. after the end of meiosis. These observations gave results which leave room for very little doubt that chromosome breaks occur in material treated during the resting stage. It seems clear, also, that chromosome splitting occurs very early during the resting stage. In the other plants it occurs quite late in the post-meiotic resting stage, and there is no doubt that chromosome breaks occur in material treated after the end of meiosis.

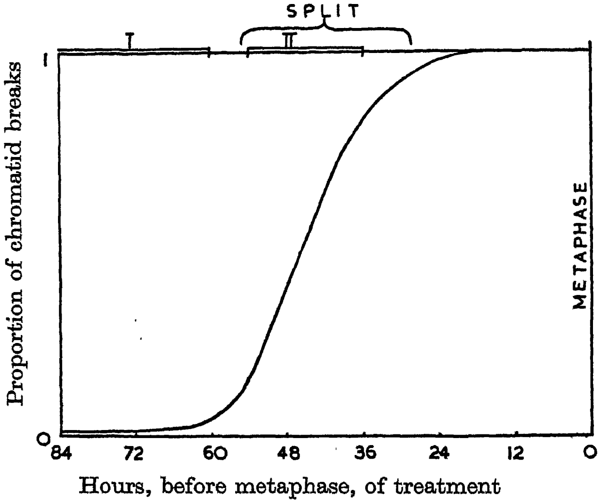

FIG. 3—Diagram illustrating the results of various X-ray experiments on the time of split, or "division", of the chromosomes. The ordinate shows the proportion of chromatid breaks, i.e. of split chromosomes. The abscissa shows the hours elapsing between treatment and fixation of the mitotic metaphases. This scale is reversed, as the late fixations give the results of irradiation early in the nuclear cycle and vice versa. The timing is that observed in the present experiments on *Tradescantia*. The time of split, as judged from this experiment, is marked at the top. The work of Riley (1936) would give the same diagram. The other regions marked at the top are the portions of the cycle observed by (I) Mather and Stone (1933) and (II) Huskins and Hunter (1935).

The immediate interpretation is quite clear. At some time during the resting stage immediately prior to pollen-grain division the chromosomes change from a state in which they act as single to X-rays to a state in which they act as double.

Parallel genetical results have been obtained. In both *Drosophila* (Patterson 1933) and *Zea* (Stadler and Sprague 1936) irradiation of the male gametes and gametophytes during resting stage produced both chromosome breaks and chromatid breaks. The former were observed by the progeny being heterozygous for a structural change. The latter were detected by the progeny being mosaic, one part of an individual having a structural change, and the other being normal. Stadler and Sprague (1936) have since shown that this same result is produced by ultra-violet irradiation. Thus in both cases some of the gametes, of the mixed sample used in genetical experiments, carry unsplit and others split chromosomes at the time of treatment.

The ultra-violet treatment produced a greater percentage of chromatid breaks than did the X-irradiation. This presumably means that pollen competition in *Zea* does not act so strongly in selecting out the grains affected by ultra-violet rays. The effect of ultra-violet treatment at the time when the chromosomes are already double is apparently not so damaging as the effect of X-rays relative to the effects of these agencies on chromosomes which have not yet split.

The genetically determined effects of X-rays and ultra-violet light are thus mutually confirmatory, and they jointly support the cytologically observed effects of X-ray on pollen-grain division.

These results may be represented in the form of a diagram (fig. 3). The ordinate shows the percentage of nuclei with chromatid breaks, i.e. in which the chromosomes were double with respect to X-rays, and the abscissa gives the time before pollen-grain mitosis of the treatment. This is shown in a reversed direction as the early fixations show the results of treatment late in the cycle and vice versa. The regions of the cycle studied by Mather and Stone and by Huskins and Hunter are marked on this diagram. The apparent contradiction of the two sets of results vanishes when they are represented in this way.

DISCUSSION

The experimental results are clear and their immediate interpretation obvious. The chromosomes change from single to double with respect to X-rays during the post-meiotic resting stage. Is this the material division of the chromosomes? It might be expected that it is because it is difficult to imagine an agency which can cause minute changes in the nature of the gene, or in the linear arrangement of the chromosome, being unable to distinguish between two separate chromatids in its action. Furthermore, if it is not the material division of the chromosomes then it is necessary to postulate either two states of doubleness, that in which the chromosomes are in fact double but act as single to X-rays and that in which the chromosome is double both materially and with respect to X-ray action, or two states of singleness, differing in whether they are single or double with respect to irradiation. Such complicating assumptions are clearly undesirable unless they are necessary to explain the data as a whole.

Further evidence on the necessity or otherwise for making these unsupported assumptions is supplied by a consideration of the general behaviour of the chromosomes.

We may infer the act of doubling from either an observable morphological change or from a functional change. The value of direct observation, and of the observational definition of doubleness, has been discussed in the opening section. The very contradictions to which direct observation has led in this connexion carry the condemnation of this method. On the other hand, there is considerable evidence available, and evidence about which there is little if any dispute, which allows of a determination of the state of *functional* doubleness, and allows, also, of determination of the time of attainment of this state.

At meiotic zygotene the chromosomes may be seen to be coming together in pairs. The individuals of a pair may be fully associated at one point and so far apart at another that there is no question of the dual nature of the subsequent pachytene association. From polyploids it is clear that the chromosomes can be associated only in pairs, and that pairs of pairs repel one another. At the end of pachytene the single chromosomes change in such a way that, at diplotene, each chromosome acts as did the pair of chromosomes at early pachytene. In particular, a pair of chromosomes at diplotene has the same appearance as four chromosomes in an autotetraploid have at pachytene. The chromosomes must be supposed to have become double. This is confirmed by the fact that, at the end of pachytene, crossing-over occurs and affects the two chromatids of each chromosome unequally.

Thus comparative cytology allows of the definition of the splitting of the chromosomes as the act of changing from a single mechanical unit in pairing to a pair of potentially distinct mechanical units. Any definition of doubling that has the chromosomes, say, double at zygotene and quadruple at pachytene has no functional meaning. The chromosomes act as a single unit at zygotene and as relatively double at diplotene. To say that a zygotene chromosome behaves as double without having a functionally single chromosome to point the comparison is merely to set up an alternative phrase for functionally single.

By comparison of meiotic and mitotic behaviour the conclusion is reached that the chromosomes are double, in this functional sense, at the earliest mitotic prophase, and remain so until metaphase. At second division of meiosis the chromosomes act as double, and hence they must be so at first anaphase. Then by comparison with first anaphase they are single at second anaphase. Similarly they are single at mitotic anaphase. Thus the chromosome cycle with respect to pairing properties is clear. In mitosis they are double at early prophase and single at anaphase. In meiosis they are single at pachytene and single at second anaphase, having been double for a large portion of the intervening period. Thus it follows that in mitosis the chromosomes have divided at the previous resting stage, and in meiosis they have failed to do so. This is the foundation of Darlington's Precocity Theory of Meiosis (1931). If further demonstration of this is necessary, it is supplied by the observations of Geitler (1932, cited by Geitler 1934). He found that in diatoms it was possible to determine the nature of a division, whether it would be mitotic or meiotic, by treatment applied during the immediately prior resting stage. This treatment then determines whether the beginning of nuclear division will precede or follow chromosome division.

This functional chromosome cycle, inferred from the external behaviour of the chromosomes, is coincident with that inferred from the X-ray results. Both types of data lead to the conclusion that the chromosomes are single at the beginning of the resting stage in any nucleus and thus divide, for any mitosis, at the immediately preceding resting stage. The X-ray results go further and allow of determination of the approximate time at which the chromosomes divide during the resting stage. Thus we have two lines of evidence giving the same result for the time of *functional* split of the chromosomes. It would need overwhelming evidence from direct observation to demonstrate the occurrence of visible splitting at a time different from functional splitting. Such evidence cannot be considered to exist at present, and the importance of pure unsupported observation must be discounted.

SUMMARY

Direct observation of the chromosomes has led to contradictory views regarding the time of the chromosome cycle at which splitting occurs. The

property of X-rays in causing chromosome breakage allows of an experimental approach to this problem. Irradiation of split, or double, chromosomes produces chromatid breaks; and the irradiation of unsplit, or single, chromosomes produces chromosome breaks. The difference between these types of rearrangement is easily visible at the subsequent metaphase.

Application of this technique to pollen-grain mitoses in three plants leads to the conclusion that splitting occurs during the post-meiotic resting stage, i.e. during the last resting stage before the mitosis observed. Genetical observations on *Drosophila* and *Zea* support the cytological results.

Considerations of chromosome pairing lead to the same conclusion as the X-ray experiments, viz. that the chromosomes divide during the resting stage immediately prior to the mitosis at which separation occurs. It is considered that, in view of this evidence relating to the functional split, the contradictory evidence relating to a visible split may be discounted.

REFERENCES

Darlington, C. D. 1931 *Biol. Rev.* **6**, 221–64.
— 1935 *Ann. Bot., Lond.*, **95**, 579–86.
— 1936 "Recent Advances in Cytology." London: Churchill.
Geitler, L. 1932 "Der Formwechsel der pennaten Diatomeen." Jena.
— 1934 "Grundriss der Cytologie." Berlin: Borntraeger.
Gustafsson, Å. 1936 *Hereditas, Lund*, **22**, 281–335.
Huskins, C. L. and Hunter, A. W. 1935 *Proc. Roy. Soc.* B, **117**, 22–33.
Lorbeer, G. 1934 *Jb. wiss. Bot.* **80**, 567–817.
Mather, K. 1934 *Hereditas, Lund*, **19**, 303–22.
Mather, K. and Stone, the late L. H. A. 1933 *J. Genet.* **28**, 1–24.
Nebel, B. R. 1936 *Genetics*, **21**, 605–14.
Patterson, J. T. 1933 *Genetics*, **18**, 32–52.
Riley, H. P. 1936 *Cytologia, Tokyo*, **7**, 131–42.
Stadler, L. J. and Sprague, G. F. 1936 *Proc. Nat. Acad. Sci., Wash.*, **22**, 584–91.
White, M. J. D. 1935 *Proc. Roy. Soc.* B, **119**, 61–84.

Proc. Roy. Soc., B, vol. 124, *Plates* 4, 5 *and* 6

ERRATA

The three plates facing page 112 and numbered 1, 2 and 3 should be numbered 4, 5 and 6 respectively.

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Foot-and-Mouth Disease and Vesicular Stomatitis
A Comparative Microscopical Study

By J. E. Barnard, F.R.S.

National Institute for Medical Research, Hampstead, N.W. 3

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[Plates 4–6]

The purpose of this paper is to describe and demonstrate the microscopical appearances, and the differences that have been observed in infective material obtained from primary vesicles, resulting from the inoculation of suitable susceptible animals with foot-and-mouth disease and vesicular stomatitis.

The pathological changes which occur in foot-and-mouth disease have been intensively studied by a number of workers. Of the established facts, one or two may perhaps be recalled with advantage in relation to the studies to be described. The primary vesicle is the chief interest since it has been the main source of the material studied, but changes occurring in distant parts of the body are not without significance. In briefest outline this is what is usually observed in the guinea-pig. During the 24 hr. immediately following inoculation of the hindfoot, local focal changes appear within the epithelial layer; steadily enlarging groups of epithelial cells degenerate and disintegrate as vesicles filled with clear fluid appear, increase in size and coalesce with neighbouring vesicles. At this stage both the vesicular fluid and the vesicular epithelium are highly infective. The blood becomes infective quite early in the disease and by the end of the second day following inoculation secondary vesicles are appearing in the epithelium of the tongue and of the soles of the forefeet. Sometimes the disease progresses till death occurs, but usually repair processes soon begin, the blood becomes non-infective, and the walls and fluid of the primary vesicles rapidly decline in infectivity. By the fourth or fifth day all the vesicles are healing. By about the end of the third week desquamation of the damaged epithelium is complete and the skin has returned to normal.

The pathology of infective stomatitis is very closely similar to that of foot-and-mouth disease. Indeed, so similar are the two diseases that it is doubtful whether, by histological methods alone, the two can be distinguished with certainty. In both, the virus has a specific affinity for epithelial cells, other cells and tissues showing only secondary reactions.

In the course of the researches to be described parallel studies have been made of the two diseases, foot-and-mouth and vesicular stomatitis, as produced experimentally in the guinea-pig. It is rarely that two diseases, so similar in their manifestations, become available for microscopical study. The necessity for controls, using that term in its generally accepted sense, has not been overlooked, but it is felt that the similar clinical pictures presented by these two diseases did in themselves provide such efficient controls as could be obtained in no other way. If it could be demonstrated that in each case a virus-like body is present in similar sites, but that there is a detectable constant difference in the picture presented, this would be of some importance. The photo-micrographs produced by suitable optical means appear to support this contention.

As a rule the guinea-pigs—the only animals referred to in the present paper—have been infected either by the method of scarification or by intradermal injection. The two methods have given identical results so far as these investigations are concerned. All observations have been made on material freshly collected from an infected animal, and in the process of making the microscopical preparation no preliminary treatment, such as is adopted in making stained preparations, has been applied. Thus the material has been examined in its fresh unaltered state. The vesicle was opened and the contained fluid collected in a small sterile tube and suitably diluted. To obtain as many free cells as possible the epidermal layer inside the vesicle was brushed gently with a camel-hair brush, and the cells thus detached were mixed in with the fluid. This fluid was diluted with Ringer's solution or buffered phosphate solution, the infected cellular elements being in suspension. The cells were allowed to sediment at about 3° C. for 2–3 hr. It was then possible to take up with a fine capillary pipette a droplet which contained a sufficient and suitable number of cells to make a satisfactory preparation. This droplet was deposited on a quartz cover-glass which was inverted on to a quartz slide. The fluid was allowed to spread out and by slight compression the space between slide and cover-glass was completely filled. This preparation of the material is a matter of considerable importance although simple enough in principle. The essential point is that the cells to be observed must be in one layer, no elements overlying one another, and yet no pressure must be applied that might cause distortion either of the cells or the virus-like bodies within them. The preparation made as described was at once sealed with paraffin wax by the method described by the author (Barnard and Welch 1934).

The preliminary observation of the preparation was made in visible light by an efficient dark-ground method of illumination which has been described already in an account of the procedure adopted for the identification of virus bodies in infectious ectromelia (Barnard 1931). This provides a means of determining the appearance of the object in terms of the visibility of the small bodies or elements of structure present in the preparation. The photomicrographs presented are all taken by means of ultra-violet light under the conditions indicated in the detailed description which follows. Two wave-lengths in the ultra-violet region have been used, a wave-length of 275 mμ for the dark-ground images and 257 mμ for those taken by transmitted light. Quartz objectives corrected specially for each of these wave-lengths have been employed, a 1·7 mm. glycerine immersion objective, effective N.A. 2·5 for dark-ground work, and a 1·66 mm. glycerine immersion objective effective N.A. 2·6 for transmitted light. There is a definite reason for using these two objectives for the purpose indicated. It is found that the transparency of many virus bodies to ultra-violet light decreases rapidly between the two wave-lengths for which these objectives are corrected and it is probable that this is a characteristic property of all animal viruses. The 1·7 mm. objective is perfectly satisfactory for dark-ground work as it is used in a wave-length of great intrinsic brilliancy which the spark-source provides, and the refractivity of the organisms in this wave-length is high enough to form a satisfactory image.

The transmitted light pictures are taken in the shorter wave-length and give as much contrast as, and indeed a general similarity to, those provided by a stained preparation of an ordinary bacillus when seen with visible light. These two methods are complementary; one provides a dark-ground picture in terms of the difference of refractivity between the organism and its surroundings, whilst the other provides an image in terms of its specific absorption. The discovery that virus bodies absorb ultra-violet radiations so completely about the region of 250 mμ, has been of great value, as it has often been possible to recognize isolated virus bodies associated with other particulate material of similar size.

To provide for the illumination of the material by the alternative methods referred to, two substage condensing optical systems have been employed. Provision is made in one form for illuminating the object by a dark-ground method in both visible and ultra-violet light. The object is therefore observed and focused in visible light and the photograph taken in ultra-violet light without alteration or readjustment of the condenser.

The other optical system consists of a glass dark-ground condenser for illuminating the object with visible light. At the centre of this illuminator and mounted concentrically with it is a quartz condenser for use with transmitted ultra-violet light. Either means can be employed by stopping out that portion of the condenser combination that is not required. These two appliances have been described elsewhere (Barnard and Welch 1936). Briefly the sequence of operations has been to find a suitable spot in the preparation in visible light, using a mercury-vapour lamp as the illuminant with a green colour screen to give monochromatic illumination, and to focus the dark-ground image as accurately as possible with a glass apochromatic glycerine immersion objective of 3 mm. focal length and N.A. 1·2. By appropriate means this objective is replaced by a quartz glycerine immersion monochromat corrected for the particular wave-length in the ultra-violet that it is desired to use, and the microscope fine adjustment altered by the amount required to give a sharp image on the photographic plate. The method of use is the same whether a transmitted light or dark-ground image is wanted, the result being determined by the type of sub-stage condenser used.

A selection of the photomicrographs, all of which are taken by the ultra-violet light method, are shown in Plates 4, 5 and 6. Plate 4 is of vesicular stomatitis and Plates 5 and 6 are of foot-and-mouth disease. The general similarity in appearance of the two organisms is apparent, although on careful comparison differences in detail will become evident.

A comparison of the photomicrographs of these two organisms shows a definite difference in size, although the variation that occurs in the appearance of each, due to changing environment, does not provide evidence by which one could be distinguished from the other with certainty. The lower limit of size of vesicular stomatitis lies between 80 and 100 mμ whilst foot-and-mouth disease goes to much smaller sizes. The lower limit suggested by the photographs for foot-and-mouth disease is about 40 mμ but there is the important difference that this organism in its most active form, where subdivision of the virus is proceeding rapidly, is a slender rod-shaped body which effectively may be smaller in diameter than this. The significant dimension of an organism is its breadth: it is in the direction of its breadth that subdivision occurs. It is of more than passing interest to note that the larger viruses so far observed are spherical in form, they only depart from this before subdivision occurs and then for but a short time as the generative process appears to be rapid. Thus vaccinia, fowl and canary pox, fowl plague, and others are normally spherical, but the smaller viruses, of which foot-and-mouth disease is an outstanding but not isolated example, tend to become rod-like although spherical forms can be seen. In foot-and-mouth disease the rod-like form is very marked, many organisms being in length at least three times the breadth. The criticism may be made that these long forms are made up of spherical or coccoid bodies, that they may be short chains. There is no microscopical evidence to support this view, as had there been any reduction in intensity of the image at any point along its greater axis the dark-ground method of illumination in ultra-violet light would be competent to show it. The optical and physical aspects of this contention cannot be discussed here, but there is ample evidence both in theory and practice to support this view. It should be remembered too that whilst the apparent length of these organisms is shown with considerable accuracy, the breadth, which is below the strict resolution limits of the objective used, will be somewhat less than the photographs show. This difference is ascertainable and amounts to as much as 20 % in the smallest elements of structure. In viewing the photographs therefore of the smallest elements, while the length may be estimated with accuracy, the actual breadth may be reduced by as much as one-fifth of the apparent breadth.

The existence of large forms of both organisms is shown by the photomicrographs, and it may be suggested that these bodies are not the same organisms as the small ones, which in the case of both viruses are always found inside the epidermal cells. The microscopical evidence appears to be against such a suggestion, as transitional forms may be seen in suitable preparations ranging from the smallest to the largest size. The large forms appear in greatest numbers in older lesions, they have not been found inside the cells, but are found in the lymph as free bodies apart from cells. This side of the subject cannot be pursued in all its implications, but it is not an isolated instance for an organism to suffer morphological change, degenerative or otherwise, a result of change of environment.

It is suggestive too that the larger organisms (see fig. 14, Plate 5) are irregular in shape, and that actively dividing forms are not seen. It is possible too, assuming that any of the bodies shown are the causative agent, that these large forms are no longer infective, as experiments by others show that infectivity is lower in the later stages of the disease.

The size of the virus bodies, as estimated by filtration experiments (Galloway and Elford 1933), is in substantial agreement with the microscopical findings in the case of vesicular stomatitis, on the assumption that filtration results are in terms of the smallest infective units. It is the end-point in filtration that determines the estimate of size. Foot-and-mouth disease does not show the same agreement between the filtration results and the microscopical images obtained, the lower limit of size suggested by filtration being about 12 mμ. It is admitted that this dimension is beyond the resolution limits of any microscope objective available at present, but it is not beyond the limits of visibility by a suitable dark-ground method. The images of the probable virus bodies as shown in the photomicrographs do not go down to the limits of size suggested by filtration. It may be that this is due to the optical shortcomings referred to, although images have been obtained of objects substantially smaller than either virus referred to herein. It is doubtful whether this point will be settled until certain optical improvements at present in course of production have come into use.

For the material with which this work was carried out I am entirely indebted to Mr. I. A. Galloway, B.Sc., M.R.C.V.S. Much of this material has become available during Mr. Galloway's own researches, but often animals have been inoculated specially for my work. It is with much appreciation that this acknowledgement is made.

REFERENCES

Barnard 1931 *Proc. Roy. Soc.* B, **109**, 360–80.
Barnard and Welch 1934 *J. R. Micr. Soc.* **54**, 29–31.
— — 1936 "Practical Photo-micrography." London.
Galloway and Elford 1933 *Brit. J. Exp. Path.* **14**, 400.

DESCRIPTION OF PLATES

Plate 4. *Vesicular Stomatitis.* × 3250

FIGS. 1 and 2—From the same sample of vesicular fluid. Fig. 1 is illuminated by transmitted light, fig. 2 by dark-ground illumination. Both these photomicrographs show the slender forms of the organism that occur in early lesions.

FIGS. 3 and 4—Similar material from another animal. The same field is shown but the comparative photographs were taken at different levels. In fig. 3, taken at a higher level, both small and large forms are seen but the mass of organisms is below the focus. In fig. 4 the mass is resolved to show the individual organisms of which it is composed.

FIG. 5—Shows variation in size and is taken from a lesion at a later stage of development. Most of the organisms are larger than in the previous photographs. This appears to occur with increasing age and when the organisms are dissociated from the cells.

FIG. 6—Shows the small forms as they exude from the cell. It will be seen that the organisms shown at the point of exact focus are perfectly defined.

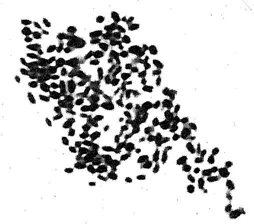

Fig. 1

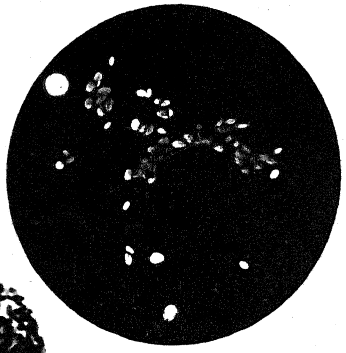

Fig. 2

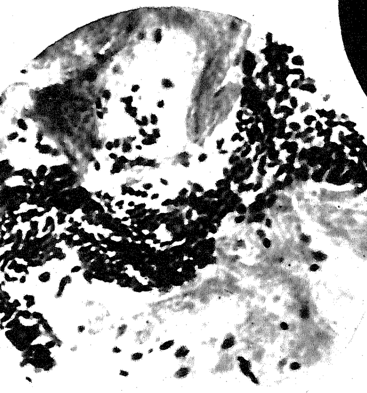

Fig. 9

Fig. 3

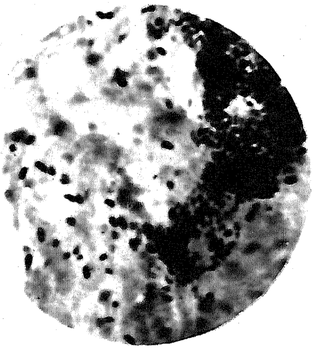

Fig. 4

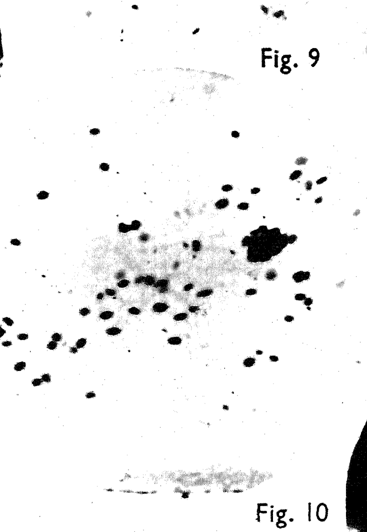

Fig. 10

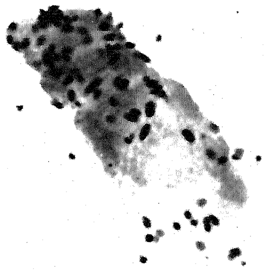

Fig. 5

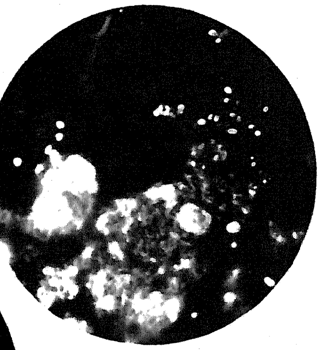

Fig. 6

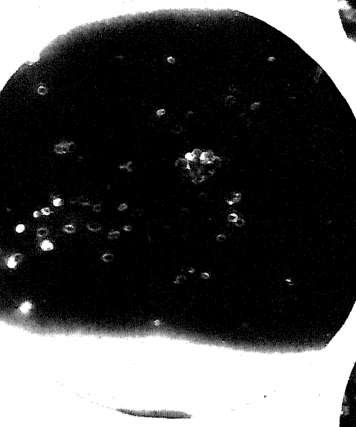

Fig. 11

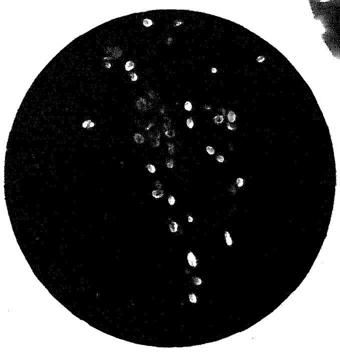

Fig. 7

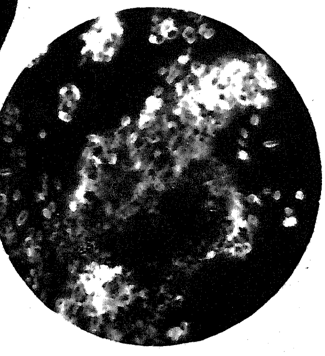

Fig. 8

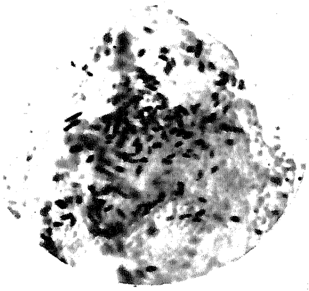

Fig. 12

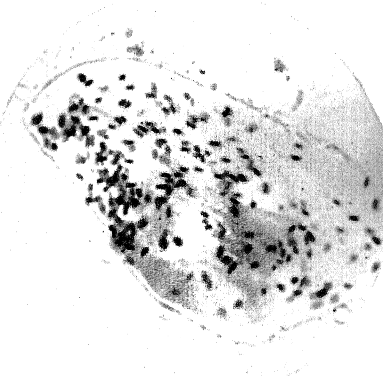

Fig. 20

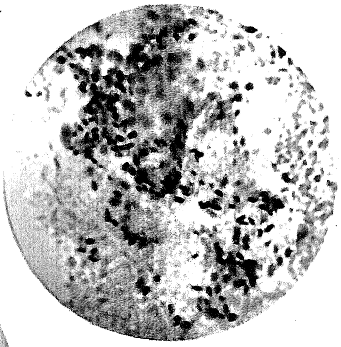

Fig. 13

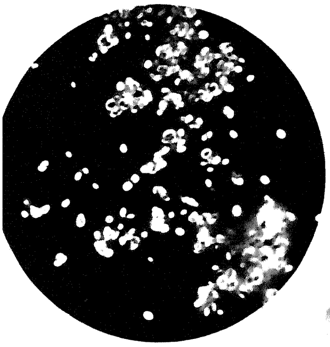

Fig. 14

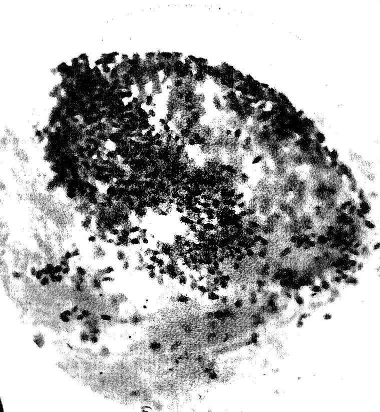

Fig. 21

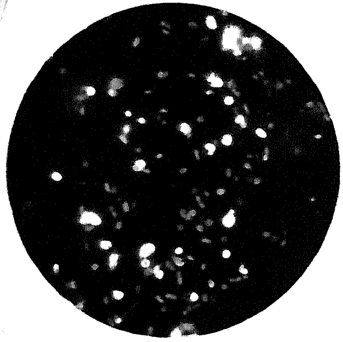

Fig. 15

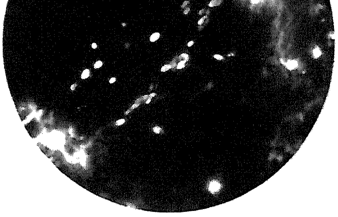

Fig. 16

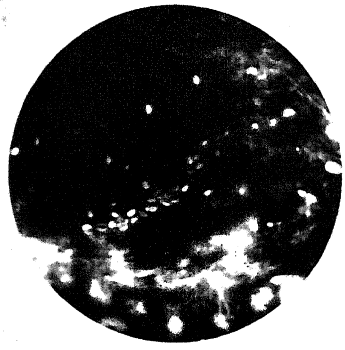

Fig. 17

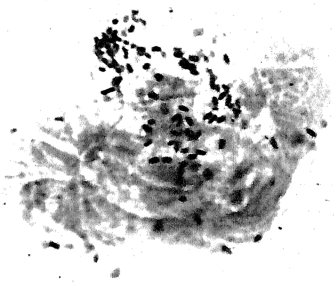

Fig. 22

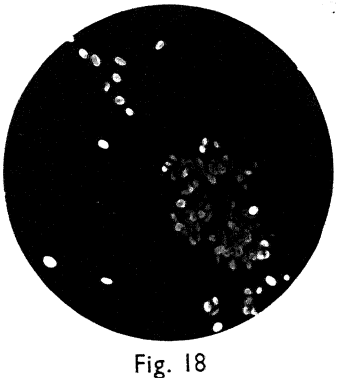

Fig. 18

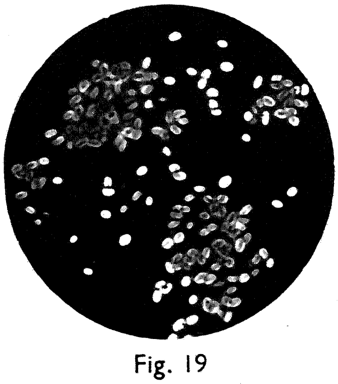

Fig. 19

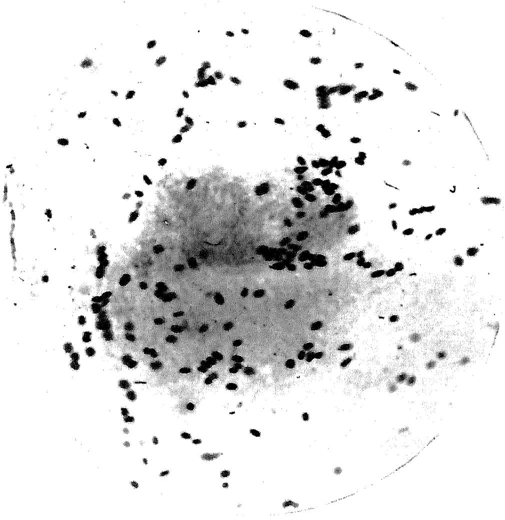

Fig. 23

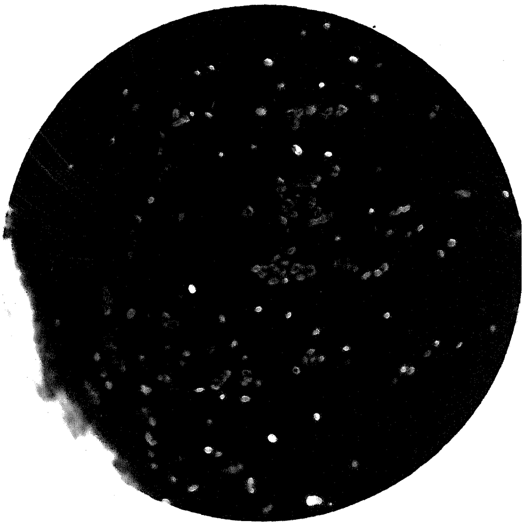

Fig. 24

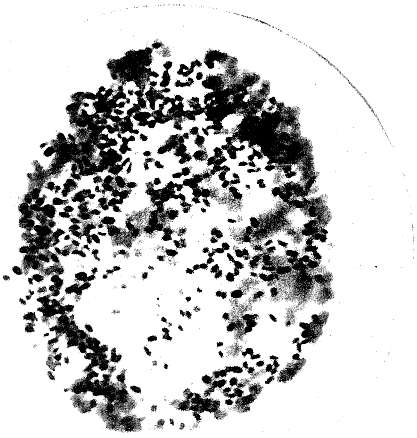

Fig. 25

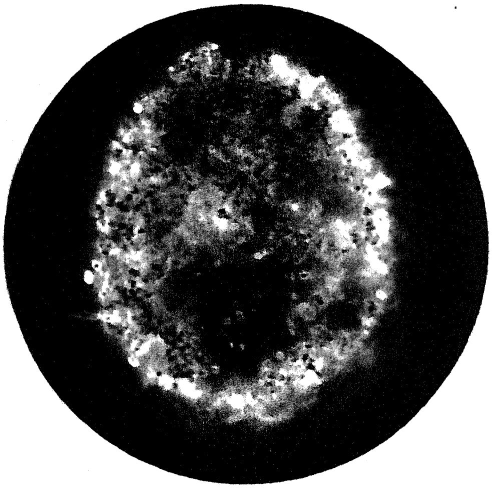

Fig. 26

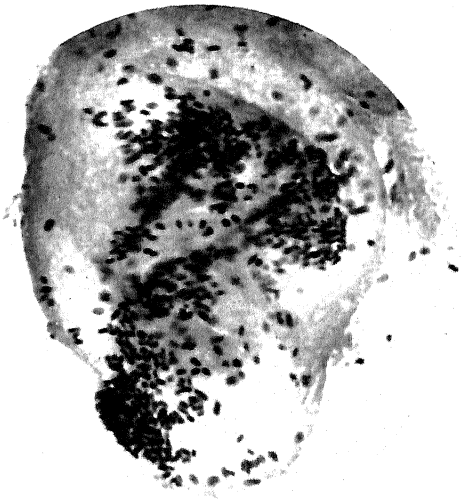

Fig. 27

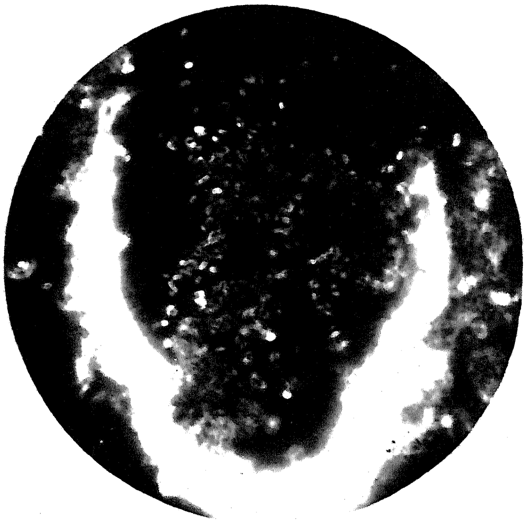

Fig. 28

FIGS. 7 and 8—Show the largest forms that have been observed in material from late lesions.

FIG. 9—A compact inclusion-like mass of organisms within the cell, only a part of which is shown. Allowing for the slight compression that is unavoidable in making the preparation, it is evident that the organisms arrange themselves with the regularity that is usually associated with compact growths of ordinary bacteria.

FIGS. 10 and 11—Comparable photographs of the same field by transmitted light and by dark-ground illumination.

Plate 5. *Foot-and-Mouth Disease.* × 3250

FIGS. 12 and 13—Photomicrographs from 36 hr. lesion from different parts of the same cell. Transmitted ultra-violet light photomicrographs showing the smallest forms observed.

FIG. 14—Dark-ground ultra-violet light photomicrograph, 48 hr. lesion showing the larger forms extraneous to the cell, mixed with smaller ones which have come from within the cell.

FIG. 15—As fig. 14, but showing a larger proportion of small forms and rod-like bodies exuded from the cell by slight compression.

FIGS. 16 and 17—Show the thin rod-shaped bodies which occur within the epidermal cells in early lesions.

FIG. 18—Shows similar slender bodies associated with larger forms.

FIG. 19—From a late lesion showing large forms only, the organisms are dissociated from the cell.

FIGS. 20, 21 and 22—Transmitted ultra-violet light photomicrographs showing organisms associated with epidermal cells. Fig. 21 suggests an appearance which is frequently observed. The organisms often entirely replace the cytoplasm and completely fill the cell. In most cases the mass is too opaque to allow a photograph to be taken, the appearance here shown is suggestive of the enormous numbers usually present. The nucleus is rarely if ever invaded.

Plate 6. *Foot-and-Mouth Disease.* × 3250

These photomicrographs enable a comparison to be made of the results obtained when the same field is photographed in dark-ground and transmitted ultra-violet light.

FIGS. 23 and 24—Show an inclination to parallelism of the virus bodies which has not been observed in the case of *vesicular stomatitis*.

FIGS. 25 and 26—Show a whole cell with contained virus bodies. This is from an advanced lesion, and some virus bodies are already beyond the confines of the cell boundary.

FIGS. 27 and 28—Part of another cell showing the virus developing in mass.

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Methods of Analysing the Heat Production of Muscle

By A. V. Hill, Sec.R.S.

From the Department of Physiology, University College, London

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The initial heat production of muscle (as distinguished from the delayed heat production) occurs so rapidly that the determination of its distribution in time, particularly in relation to contraction, relaxation and the performance of mechanical work, requires special methods of analysis (see e.g. Hartree 1933). The only means hitherto found satisfactory of measuring muscle heat production has employed a thermopile and galvanometer. For the rise of temperature involved the e.m.f. developed by the thermopile is only a few microvolts, and it has not been possible to devise any really satisfactory method of amplification: the current has had to be read directly by the galvanometer. Since the current was small the galvanometer had to be sensitive, and therefore comparatively slow. This introduced the first element of delay in the measurement of the heat, that due to the galvanometer. The second was due to the thermopile, for all thermopiles hitherto employed have been rather slow in reaching the temperature of the muscle. Moreover, their heat capacity has been so large that the sensitivity of the system has been considerably less than that calculated simply from the constants of the apparatus.

For all these reasons the analysis and the calibration have been performed by heating the muscle electrically during a known short interval, or "instantaneously", and finding numerically what distribution of such heat-pulses would give the same deflexion, in time and amplitude, as the heat produced by the stimulated muscle. The method is laborious and there are various objections to it, particularly (i) the requirement that the muscle must be of uniform cross-section if it is to be uniformly heated (see Hill 1931*b*, p. 144, Appendix II), (ii) the need of very good electrical insulation, in order to avoid leaks, and (iii) the danger of non-uniform heating in the immediate neighbourhood of the electrodes leading current to the muscle. Within limits, however, the method has fulfilled its purpose, and all significant results on the time-course of the heat production of muscle (and nerve) have been obtained with it.

In the present paper a method is described in which the heat capacity of the thermopile, and its lag in reaching the temperature of the muscle, are made very small. It will still be necessary to use a "heating control" for the analysis of the delayed heat production, since the thermo-e.m.f. of the thermopile is bound to diminish with time, owing (i) to cooling of the muscle and (ii) to warming of the "cold" junctions by conduction: and no simple method of allowing for these is available. For the initial heat, however, the use of a thermopile with rather long thin wires of low thermal conductivity (e.g. manganin and constantan) prevents any significant loss of thermo-e.m.f. in the few seconds covering the initial heat, and the new method, which is simpler and more accurate than the old, is available.

Recent improvements in galvanometers, on the lines of the Moll "microgalvanometer", have made it possible to obtain sufficient sensitivity for myothermic experiments with a total (undamped) period of 0·20–0·35 sec. The construction and performance of such instruments are described by Mr. A. C. Downing in Appendix I below. With so quick a galvanometer it is not difficult to analyse a photographic record so as to get a comparatively true picture of the e.m.f. of the thermopile at all times during and after a contraction. The method of analysis is described in Appendix II. Unless, however, the thermopile is very rapid, its e.m.f. does not represent sufficiently nearly the temperature of the muscle on it and nothing is gained. Mr. Downing, however, has succeeded in making thermopiles so thin, and of heat capacity so small, that their e.m.f. follows with insignificant lag the temperature of the muscle. Their construction is described by him in Appendix III. Their couples are of 42 s.w.g. constantan and manganin wire, rolled to about 15μ thickness. Their insulation is of mica with a minimal amount of bakelite varnish: its resistance need not be very high (though it may in fact be hundreds of megohms after prolonged soaking), since electrical heating is now unnecessary. The thinnest thermopile hitherto constructed is 65μ thick, and is equivalent in thermal capacity to about 40μ of muscle; it is expected that one of 45μ, equivalent to about 28μ of muscle, will shortly be available. Part I of this paper describes a mathematical and experimental examination of the time taken by such thermopiles to reach the temperature of the muscle.

During the course of this investigation it was realized that, in experiments in which a muscle is allowed to shorten, an error may result, however constant and uniform the temperature of the apparatus, from the fact that different parts of the muscle itself may be at different temperatures. This error is one which has dogged the footsteps of those who have worked on muscle heat (cf., for example (Hill 1913), the *negative Wärmeschwankungen* of the earlier German workers): but it had been supposed that it could be avoided by keeping the thermopile and its chamber at a very uniform and constant temperature. It had not been realized that the heat production of the muscle itself, either the resting heat, or the recovery heat and the undissipated remainder of the initial heat of previous contractions, might make parts of the muscle not lying on the thermopile appreciably warmer than parts lying on it. The thermal conductivity even of the thinnest thermopile is considerably greater than that of air: so the part of the muscle on the thermopile must lose its heat faster, and so be cooler, than the rest.

The difficulty of fitting into any clear scheme the results obtained by many investigators* on the energy exchanges of muscles shortening, lengthening, doing mechanical work, or having work done on them, encouraged one to examine the matter further. There were clear signs of thermodynamic relationships in the various results, yet no consistent picture of them could be formed. Possibly some at least of the results were inaccurate, and if errors could be cleared away a much more consistent picture might emerge.

One possible method of detecting the error, if it existed, was to use a thermopile with three galvanometer leads, *Dd*, *Ee* and *Ff* (fig. 4 below). The part between *F* and *E* was nearer the fixed end of the muscle, the part between *E* and *D* nearer the movable end. The part *ED* was long enough to ensure that when the muscle shortened any part of it which came on *FE* had previously been on *ED*, and so had been subjected to identical conditions of heat loss. The record, therefore, from *FE* would not be affected by the error in question, that from *ED* would be. An experimental comparison of the records from *FE* and from *ED* respectively, which were exactly similar in isometric contractions, showed that grave errors affected the results when shortening was allowed. These errors must have occurred in all shortening experiments in which a thermopile was not "protected" by a sufficient length of similarly conducting material beyond it. They, and the method of avoiding them, are described in Part II.

In dealing with muscles actually shortening, or in comparing muscles at different lengths, another difficulty in the earlier method was that the "heating control" curve and the "calibration number" depended on the thickness of the muscle (see e.g. Hill 1925, p. 242): the thermal capacity of the thermopile was not nearly small enough to be neglected and naturally the muscle when shorter, and therefore thicker, warmed it more. It was difficult to allow for this accurately. With a very small heat capacity, however, in the thermopile this trouble does not occur: no "heating control" or "calibration" need be made, the temperature of the muscle being given closely enough by the e.m.f. of the thermopile. Thus with a thin thermopile of the kind described in Appendix II, "protected" by a suitable extension of similar thermal conductivity, it should be possible, without error or difficulty, to measure, and to determine the time-course of, the heat production of muscles of any convenient shape, and undergoing any required manoeuvres of shortening, lengthening or doing mechanical work.

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* Fenn (1923, 1924), Azuma (1924), Hartree (1925), Wyman (1926), Hartree and Hill (1928 *a* and *b*), Hill (1930, 1931 *a*), Cattell (1932), Feng (1932 *b*).

In using such thermopiles, or indeed any others, one special precaution is worth emphasizing: the contact of the muscle with the face of the thermopile should be as uniform as possible throughout contraction, in particular the surface of the instrument should be quite flat and parallel to the line of pull of the muscle. A groove built up with insulator to confine the muscle more precisely to the region of the hot functions, a little loop of wire to guide the thread from the tendon, an accurate adjustment of the clamp: such simple devices and precautions are of more than minor importance in securing reliable results. They and others like them will occur to the skilled experimenter once he realizes the necessity of ensuring extreme uniformity in the thermal contact of muscle and instrument.

PART I—THE FLOW OF HEAT FROM MUSCLE TO THERMOPILE

For myothermic experiments it is best that the muscle should lie as a uniform sheet on the face of the thermopile: the sartorius of the frog and the retractor of the pharynx of the snail are of suitable shape. For quickness of response it is very important that both sides of the thermopile should be covered by muscle: if only one side is covered the time taken to reach any given degree of temperature equalization will be four times as long. Moreover, the heat capacity of the thermopile is twice as great with respect to one muscle as it is with respect to two. We will assume, therefore, that a muscle lies on each face of the thermopile: and since the thickness of any suitable muscle is rather small compared with its width, we will assume the muscle to be an infinite plane sheet of thickness a on each side of the thermopile, and the latter to be an infinite plane sheet of thickness $2b$. If a single muscle is used, on one face only, the equations are identical save that b is now the full thickness and not half the thickness.

It is impossible, with a very thin thermopile, to measure experimentally the speed with which the temperature rises: hence the need of mathematical treatment. With a thicker thermopile, however, the speed *can* be measured, and compared with that calculated mathematically. The agreement is found to be good: the assumptions, therefore, of the mathematical treatment are verified, and the latter can be used to determine the speed of the thinner thermopiles which is too high for experimental measurement.

Let K be the thermal conductivity of the muscle, c its specific heat, ρ its density. Then for plane conduction,

$$\frac{dy}{dt}=\frac{K}{c\rho}\frac{d^2y}{dx^2},$$

where y is temperature, t time and x distance measured perpendicular to its surface from the middle plane of the thermopile. Writing $k=K/c\rho$ this becomes

$$\frac{dy}{dt}=k\frac{d^2y}{dx^2}. \tag{I}$$

Assume that all the constants are the same for thermopile (which is largely insulation) as for muscle: this will be justified approximately below. Then equation (I) holds throughout the system.

It is required to determine how rapidly the thermopile approaches the temperature of the muscle when the latter is suddenly warmed by an "instantaneous" production of heat. The statement of the problem is simpler if we suppose that heat is suddenly produced in the thermopile which then cools by conduction to the muscle. It is easy, in the end, to return to the original problem.

First regard the muscle as infinitely thick and the thermopile as infinitely thin. A solution of equation (I), as can be verified by differentiation, is

$$y=\frac{A}{\sqrt{t}}e^{-x^2/4kt}.$$

This fulfils the conditions:

- (a) that at $t=0$, $y=0$ everywhere except at $x=0$;
- (b) that at $t=\infty$, $y=0$ everywhere;
- (c) that at $x=\infty$, $y=0$ always.

The total amount of heat, per unit of surface, is $\int_{-\infty}^{\infty} yc\rho dx$, which by integration is $2c\rho A\sqrt{(k\pi)}$: i.e. the heat content is constant, viz. that liberated instantaneously at $x=0$ at zero time. Thus the equation represents the distribution of temperature throughout the muscle at time t after an instantaneous liberation of heat in a very thin layer at $x = 0$. If the layer in question be the thermopile, and if the heat be that required to warm it through y_0 degrees, the heat is $y_0 2bc\rho$: equating this to $2c\rho A\sqrt{(k\pi)}$ we find $A = y_0 b/\sqrt{(k\pi)}$, and the solution becomes

$$y = \frac{y_0 b}{\sqrt{(k\pi t)}} e^{-x^2/4kt}. \tag{II}$$

This equation is generally accurate enough for practical purposes: the temperature of the thermopile is that at $x = 0$ and is therefore

$$y = \frac{y_0 b}{\sqrt{(k\pi t)}}. \tag{III}$$

We see that the time required to attain any required degree of approximation to the temperature of the muscle is proportional to the *square* of the thickness of the thermopile. This is the reason (i) why a thin thermopile is so much more rapid than a thick one, and (ii) why it is important to have a muscle on each face.

We can pass now to the case of a muscle of infinite thickness and a thermopile of finite thickness. Replacing $2b$ by $\delta\xi$ in equation (II) and x by $(x-\xi)$, clearly

$$y = \frac{y_0 \delta\xi}{2\sqrt{(k\pi t)}} e^{-(x-\xi)^2/4kt}$$

represents the distribution of temperature, in space and time, after a sudden liberation of heat at $t = 0$ and $x = \xi$. Thus if, at $t = 0$, heat be produced throughout the thermopile in amount sufficient to raise its temperature uniformly to y_0, the resulting distribution at time t will be

$$y = \frac{y_0}{2\sqrt{(k\pi t)}} \int_{-b}^{+b} e^{-(x-\xi)^2/4kt} d\xi. \tag{IV}$$

From equation (IV) we can pass to the general case of a muscle and a thermopile both of finite thickness. If the muscle ends and is insulated by air at $x = \pm(a+b)$, the essential condition is that no heat can flow out or in there. This condition is met by putting images at $\pm 2(a+b)$, $\pm 4(a+b)$, $\pm 6(a+b)$, etc. Equation (IV) then becomes

$$y = \sum_{n=-\infty}^{n=+\infty} \frac{y_0}{2\sqrt{(k\pi t)}} \int_{-b}^{+b} e^{-\{x-\xi+2n(a+b)\}^2/4kt} d\xi. \tag{V}$$

This is the complete solution. We are interested, however, in the temperature of the thermopile, which since the wires are always thin is effectively that at $x = 0$. Thus the temperature of the thermopile is

$$y = \sum_{-\infty}^{+\infty} \frac{y_0}{2\sqrt{(k\pi t)}} \int_{-b}^{+b} e^{-\{\xi-2n(a+b)\}^2/4kt} d\xi. \tag{VI}$$

Consider first the term for which $n = 0$, viz.

$$\frac{y_0}{2\sqrt{(k\pi t)}}\int_{-b}^{+b} e^{-\xi^2/4kt}\,d\xi.$$

The part inside the integral can be expanded as a fairly rapidly converging series and integrated and we get,

$$y = \frac{y_0 b}{\sqrt{(k\pi t)}}\left[1 - \frac{1}{3}\frac{b^2}{4kt} + \frac{1}{5}\frac{1}{2!}\left(\frac{b^2}{4kt}\right)^2 - \dots\right].$$

This has been calculated as a function of kt/b^2, including all significant terms and over the whole range that matters, for $y_0 = 1$, in Table I.

TABLE I—TEMPERATURE OF THERMOPILE AFTER INSTANTANEOUS HEATING: MUSCLE THICKNESS ASSUMED INFINITE

kt/b^2	0·125	0·167	0·250	0·350	0·500	0·750	1·00	1·25
y	0·953	0·917	0·843	0·767	0·682	0·585	0·521	0·473
kt/b^2	1·50	2·0	3·0	4·0	6·0	9·0	10	16
y	0·436	0·383	0·317	0·276	0·227	0·186	0·177	0·140
kt/b^2	20	25	36	49	64	81	100	
y	0·126	0·113	0·094	0·081	0·070	0·063	0·056	

The thermopile is supposed to be suddenly warmed at $t = 0$ and y is its temperature thereafter.

Consider now the n-terms. For $n = \pm 1$ the integral is $\int_{-b}^{+b} e^{-(a+b)^2/kt}\,d\xi$, since ξ (which is never greater than b) is small within these limits compared with $2(a+b)$. It is assumed that the thermopile is fairly thin compared with the two muscles. Thus the $n = \pm 1$ terms taken together provide the correction $\frac{2y_0 b}{\sqrt{(k\pi t)}}e^{-(a/b+1)^2/(kt/b^2)}$. Similarly the $n = \pm 2$ terms provide $\frac{2y_0 b}{\sqrt{(k\pi t)}}e^{-4(a/b+1)^2/(kt/b^2)}$, the $n = \pm 3$ terms $\frac{2y_0 b}{\sqrt{(k\pi t)}}e^{-9(a/b+1)^2/(kt/b^2)}$, etc. The total correction is the sum of these terms and has been calculated (Table II) for various values of kt/b^2 and of a/b. We see that, except for long times and for very thick thermopiles (a/b small), the correction to allow for the thickness of the muscle is small or negligible.

The last column of Table II represents the correction for muscle thickness after complete redistribution of the heat. We suppose that the heat initially set free in thickness $2b$ of thermopile is uniformly distributed in the end in $2a$ of muscle and $2b$ of thermopile, so the final temperature is $y_0 b/(a+b)$. Reverting now to the case of a muscle suddenly warmed and a thermopile following it, we must add the correction of Table II to the y of Table I, and subtract the sum from unity: the difference is the rise of temperature of the thermopile. The more pertinent quantity, however, is the rise of temperature of the thermopile as a fraction of that finally attained: this is found by multiplying the quantity last calculated by $(1+b/a)$. Examples are given below.

TABLE II—CORRECTION TO BE ADDED TO y IN TABLE I TO ALLOW FOR THE FINITE THICKNESS OF THE MUSCLE

$kt/b^2=$	1	4	6	9	16	25	36	49	64	81	100	∞
$a/b=5$	0	0	0·001	0·007	0·030	0·054	0·073	0·086	0·096	0·104	0·110	**0·167**
$a/b=10$	0	0	0	0	0	0·002	0·007	0·014	0·021	0·035	0·045	**0·091**
$a/b=20$	0	0	0	0	0	0	0	0	0	0·001	0·001	**0·048**
$a/b=30$	0	0	0	0	0	0	0	0	0	0	0	**0·032**

The values at $t=\infty$ represent the final correction after complete equalization of temperature.

The absolute values of the constants. (1) *Muscle*—Muscle is about 80% water and the remainder is chiefly protein. The thermal conduction of muscle cannot be far from that of its water plus that of its protein. According to tables the K of water is 14×10^{-4}, that of hair, silk and leather 5, 9 and 4×10^{-4} respectively. Assume $K=6\times10^{-4}$ for the protein of muscle: the exact value makes little difference. Then for muscle $K=(0{\cdot}8\times14+0{\cdot}2\times6)\times10^{-4}=12{\cdot}4\times10^{-4}$. Typical values for muscle of ρ and c are (Hill 1931*a*), $\rho=1{\cdot}054$, $c=0{\cdot}872$; therefore $\rho c=0{\cdot}92$, and $k=K/\rho c=1{\cdot}35\times10^{-3}$.

(2) *Thermopile*—The thermopiles chiefly considered consist of 15μ wires covered with mica. In conduction and heat capacity the mica is much more important than the wire. For *mica* perpendicular to the cleavage planes $K=1{\cdot}8\times10^{-3}$: also $\rho=3$, $c=0{\cdot}21$: hence $\rho c=0{\cdot}63$, $k=2{\cdot}9\times10^{-3}$.

For *manganin*, $K=52\times10^{-3}$, $\rho=8{\cdot}5$, $c=0{\cdot}097$: hence

$$\rho c=0{\cdot}82,\quad k=63\times10^{-3}.$$

For *constantan*, $K=54\times10^{-3}$, $\rho=8{\cdot}9$, $c=0{\cdot}098$: hence

$$\rho c=0{\cdot}87,\quad k=62\times10^{-3}.$$

For the thin thermopiles here considered temperature equalization in the instrument itself is extremely rapid, so rapid that its actual value of k scarcely affects the result except at very short times. The conduction which chiefly affects the result is that in the muscle: the temperature of a very thin thermopile lying between two muscles rises practically as a whole, as heat is conducted up to it from the muscle. The thickness of the thermopile is of consequence almost only because of its heat capacity, the thicker thermopile taking more heat and therefore a longer time. Thus the fact that k for mica is about twice that for muscle has little effect: what effect it has is slightly to quicken conduction, and so to make the instruments slightly better than here calculated.

The fact, however, that the thermal capacity of the thermopile is less than that of muscle has a very distinct advantage. For example, with a thermopile consisting of 15μ of wire with 20μ of mica on each face the mean ρc is $(15 \times 0{\cdot}85 + 40 \times 0{\cdot}63)/55 = 0{\cdot}69$. The ρc of muscle being 0·92 the 55μ of thermopile is equivalent only to 38μ of muscle. In this case we may take $2b$, the equivalent thickness of the thermopile, as 38μ instead of 55μ. Since $(38/55)^2$ = about $\frac{1}{2}$, the time to any specified degree of temperature equalization is reduced to about one-half, chiefly by the lower thermal capacity of the mica.

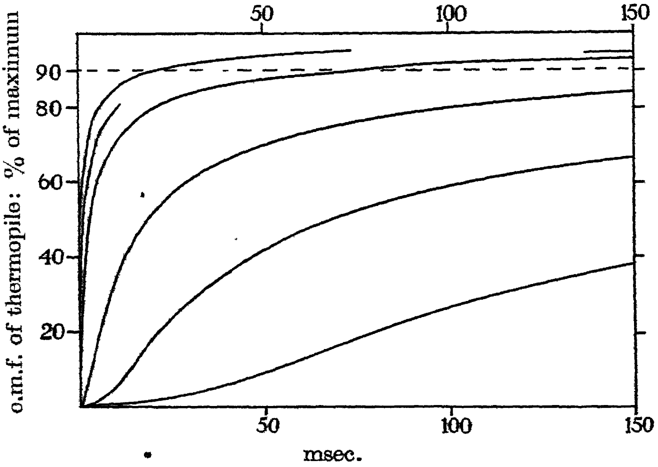

FIG. 1—The rise of temperature of a thermopile after instantaneous heating of the muscle on it. Calculated as described in text. The muscle is assumed to be 0·1 cm. thick on both faces of the thermopile, the thermopile to have an equivalent half thickness (in cm.) given by following the values of b (from below upwards): 0·02, 0·01, 0·005, 0·002, 0·0014, 0·0001.

Examples—Fig. 1 shows the calculated behaviour of six thermopiles after the muscle on them has been suddenly warmed. The muscle is supposed to be 0·1 cm. thick on each face: b is the equivalent half thickness of the thermopile in cm., i.e. the thickness of muscle which would have the same thermal capacity as half the thermopile. The curve for $b = 0{\cdot}02$ is calculated for a very slow instrument actually in use, of which an experimental test is shown in fig. 2. The curve for $b = 0{\cdot}002$ is calculated for an instrument rather thinner than one recently constructed by Mr. Downing, which has 15μ wires and 25μ mica on each face. This proved much too fast for an accurate experimental test. The curve for $b = 0{\cdot}0014$, which is shown also on a larger scale of time in fig. 1*a*, approximately represents a very thin instrument now under construction. It should give 90 % of its full e.m.f. within 37 msec., 94 % within 100 msec. It should be rapid enough for a good analysis in units of 0·1 sec. or less.

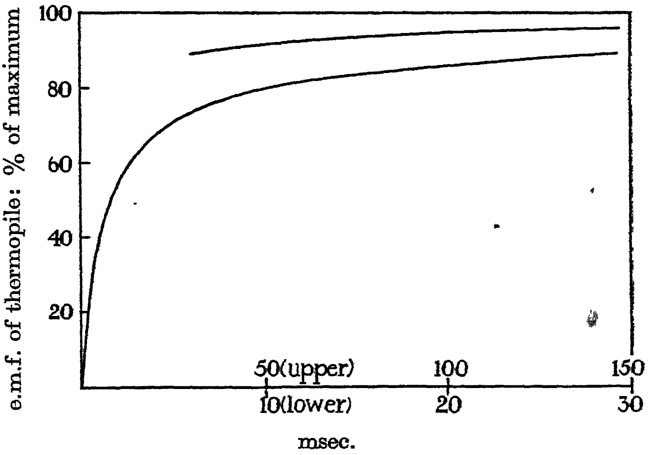

FIG. 1*a*—As fig. 1, curve for $b = 0{\cdot}0014$ cm. shown on enlarged time-scale. This represents the thinnest thermopile which it is regarded as practicable, at present, to construct.

The accuracy of the calculation has been tested in several experiments with slower thermopiles. In that of fig. 2 a thermopile 0·53 mm. thick, with a calculated b of 0·02 cm., had a pair of dead muscles each about 0·1 cm. thick on it, which were heated by a condenser discharge of $0{\cdot}7\mu$F, 315 V. It was connected to a galvanometer of period 0·22 sec. and the deflexion was analysed in units of 0·04 sec. by the method described in Appendix II, to give the e.m.f. of the thermopile. The results of the analysis (which is the actual one of Table VI, Appendix II) are shown in square blocks, the calculated rise of temperature by the curve. The agreement is satisfactory. In the experiment of fig. 3 a thermopile about 0·18 mm. thick, with a calculated b of 0·006 cm., was similarly tested. The calculated curve agrees satisfactorily with the results of the analysis in 0·02 sec. The agreement of figs. 2 and 3 is not due to any adjustment of constants, for all were measured directly: the calculation, therefore, is verified, and may be applied to the thinner thermopiles which are too fast for direct measurement of their speed.

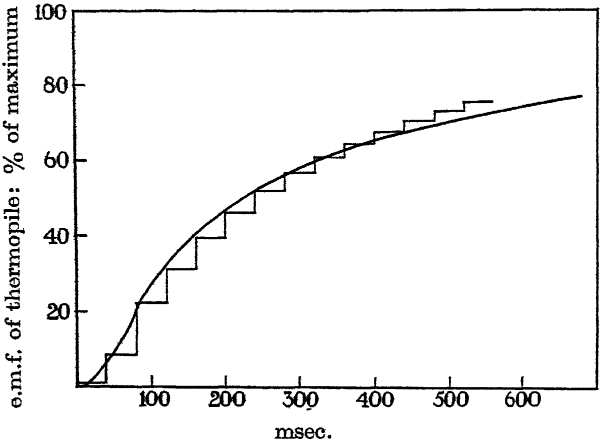

FIG. 2—Comparison of calculated with observed rise of temperature of a thermopile 0·053 cm. thick after "instantaneous" heating of a muscle on it. See text.

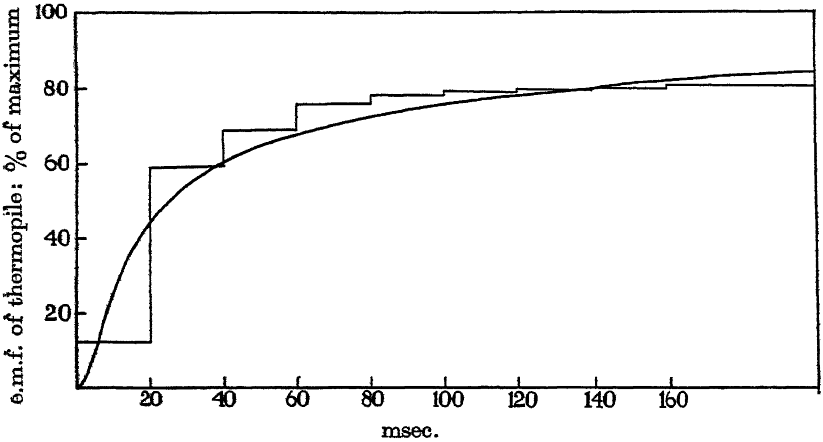

FIG. 3—Comparison of calculated with observed rise of temperature of a thermopile 0·018 cm. thick after "instantaneous" heating of a muscle on it. See text.

Discussion—The curves of figs. 1 and 1*a* show that thermopiles can be constructed which give nearly their full e.m.f. in 0·1 sec., which is short enough for an analysis of the heat production of frog's muscle at 0° C. They give 70 % of their e.m.f. in a few milliseconds, and could be used with a much shorter interval than 0·1 sec. without considerable error. They have still a slight lag, but this could not really be avoided by any means whatever: even if the thermopile were indefinitely thin there would still be a film of Ringer's fluid between muscle and thermopile, and very probably a layer of connective tissue or of imperfectly contracting fibres, and these would still provide a lag as great as that of a thermopile with $b = 14-20\,\mu$. Thus the method of analysis described should give as accurate results as the nature of the material would in any case allow.

Calibration—The large heat capacity of former thermopiles made it necessary to calibrate the apparatus in absolute units by a known amount of energy liberated electrically in the muscle: and since the calibration depended on the ratio of the heat capacities of muscle and thermopile it was affected by the thickness, and therefore by the length, of the muscle. The calibration added greatly to the labour of experimentation, and its accuracy, for a variety of reasons, was not very high. With the thin thermopiles here described the heat capacity is small compared with that of the muscle: e.g. a thermopile with $2b = 0{\cdot}005$ cm. has only $2\frac{1}{2}$ % of the heat capacity of a 1 mm. muscle on each face of it, and even that could be allowed for approximately while the effect of length must be negligible. Thus it should be possible, from the constants of the apparatus, to express all deflexions rather accurately in degrees Centigrade. Knowing the specific heat of muscle (Hill 1931*a*) the rise of temperature gives heat per gram: knowing the total weight of muscle we can calculate heat in absolute units for comparison, for example, with work done.

A further complication with some earlier thermopiles was that, owing to the high thermal conductivity of the iron or silver wire used in them, or to the short distance from hot to cold junctions, the e.m.f. fell off so rapidly that the maximum galvanometer deflexion was appreciably diminished (see e.g. Hartree 1931, p. 22: the "heating control" had fallen appreciably by 1·5 sec.). This can be avoided by using wire of low thermal conductivity (manganin-constantan, thermo-e.m.f. about 40×10^{-6} V/1° C.), by increasing the distance from hot to cold junctions, and (to some degree) by "loading" the latter with solder to prevent them from warming up so quickly by conducted heat. The thin thermopiles here described give a maximum e.m.f. which is effectively constant for several seconds, and this trouble does not occur. It is avoided, however, at some expense of sensitivity, since the electrical conductivity runs parallel to the thermal conductivity and a thermopile which better preserves its maximum e.m.f. inevitably has a higher electrical resistance. A minor disadvantage is that the slower heat loss means that the instrument takes longer after one observation to settle to a zero steady enough for another. In the design of a thermopile for any purpose a balance must be struck between these several factors.

PART II—AN ERROR IN THE MEASUREMENT OF THE HEAT PRODUCTION OF MUSCLES ALLOWED TO SHORTEN, AND A "PROTECTED" THERMOPILE FOR AVOIDING IT

A muscle at rest produces heat, and in consequence its temperature is above that of its surroundings. The steady difference of temperature finally reached represents a balance between heat production and heat loss. A muscle on a thermopile loses heat largely by conduction laterally along the wires and insulator, but partly by conduction and convection in the gas around it, and to some degree by evaporation—due to the warmer muscle having a slightly higher vapour pressure than the Ringer's fluid in which it was previously soaked. The steady temperature, shown by a steady deflexion of the galvanometer, can be used as a measure of the rate of resting heat production (Hill 1928; Feng 1932*a*; Beresina 1932; v. Euler 1935; Solandt 1936). The reading is less the greater the thermal conductivity of the thermopile.

For a muscle in air or oxygen the heat loss is only through the gas, and the final steady temperature is higher than in a muscle on a thermopile in which heat loss occurs through the wires as well. Thus, owing to the resting heat production, there must arise a difference of temperature between those parts of a muscle which are off a thermopile and those which are on it. When, therefore, the muscle shortens, the heat observed is greater than it ought to be, when it relaxes and its warmer parts move away the heat observed is too small. In respect of the total initial heat the two effects tend to balance, but not exactly, since some of the heat of the warmer part of the muscle is left behind in the thermopile when the muscle relaxes.

The effect is not small. Even with a thermopile of high thermal conductivity (e.g. Hill 1928) the steady deflexion at room temperature due to the resting heat production is about one-half of that caused momentarily by a muscle twitch: with a thermopile of low thermal conductivity (e.g. v. Euler 1935; Solandt 1936) it is considerably greater. The part, therefore, on the thermopile must be 0·001–0·006° C. warmer than its surroundings, so that the part off the thermopile, which is cooled only through the air, may be anything up to 0·01° C. warmer. This warmer part, coming on to the thermopile in contraction, would cause a considerable disturbance. At 0° C. the rate of resting heat production is less, say $\frac{1}{4}$, so the error is correspondingly reduced. Fortunately the time course of the heat production in muscles allowed to shorten (Hartree 1925; Hartree and Hill 1928*b*) has always been determined at 0° C., and so far as the resting heat production was responsible the error must have been rather small.

More serious, however, than the resting heat, under most experimental conditions, is the heat left over from previous contractions. It is seldom possible for an observer to wait 15–20 min. between successive readings,

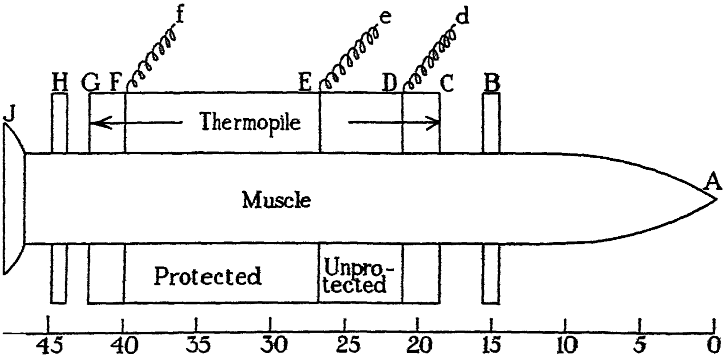

FIG. 4—Thermopile for studying error due to movement. *A*, tendon of muscle; *J*, bone; *B*, *H*, stimulating electrodes; *CG*, thermopile; *CD*, *FG*, "dummy" portions; *DE*, "unprotected" portion; *EF*, "protected" portion; *Dd*, *Ee*, *Ff*, leads to galvanometer.

and unless he does so the muscle will still be recovering (if oxygen be present) and producing recovery heat. This will add to the error due to the resting heat. Moreover, the initial heat requires several minutes for its loss by conduction, etc., through air, so that unless observations are made at long intervals the undissipated remainder of the initial heat of previous contractions may add to the disturbance. This last trouble may be very serious, as is shown below, and is not avoided by working at a low temperature.

The error due to movement was explored by a special thermopile shown diagrammatically in fig. 4. On this was fixed, at 19° C., a pair of sartorii of a Hungarian frog: *A* is the tendon, *J* the pelvic bone. *B* and *H* are the stimulating electrodes. *CD* is a "dummy" portion of thermopile, containing wires except close to *C*, but without a connexion at *C* to the galvanometer.

FG is a similar "dummy" portion. *CD* and *FG* are similar to the main thermopile *DF*. The thermopile is about 0·125 mm. thick and has rolled wires (constantan-manganin) about 25μ thick. It has three leads, *Dd*, *Ee* and *Ff*, so that the whole thermopile, or either of its portions *DE* and *EF*, can be separately connected to the galvanometer.

The thermopile *DE* is "unprotected" against any considerable shortening of the muscle: i.e. when the muscle shortens a portion of it previously off the thermopile comes on to it. Against slight shortenings, as in a nearly isometric contraction, it is "protected" by *CD*. The thermopile *EF* is completely "protected" by *CD* and *DE* at one end and by *FG* at the other. The muscle could not shorten so much that a part of it came on to *EF* which was not previously on *CE*: while in any slight movement at the bone end no new part of the muscle ever came on to *EF* which had not previously been on *FG*. Thus the "protected" thermopile *EF* should be completely free from the error considered: the "unprotected" thermopile *DE* should be largely affected.

The thermopiles were used with a galvanometer of period 0·22 sec. *EF* had more couples than *DE* and so gave a larger deflexion: the *shape*, however, of the record obtained by stimulating, and not its magnitude, alone was considered. *EF* had a higher resistance than *DE*, so when the latter was used a resistance (equal to the difference) was introduced to maintain critical damping.

The similarity of the two thermopiles, *DE* and *EF*, in use was confirmed by making several photographic records from each for an approximately isometric contraction (0·2 and 0·3 sec. tetanus). The deflexions were practically identical in shape, showing (i) that the thermopiles, and the portions of muscle on them, were similar, and (ii) that *DE* was sufficiently "protected" for an isometric contraction.

With isotonic contractions, however, the deflexions obtained from the "protected" and the "unprotected" thermopiles were quite different. Even after a very long rest, to allow for the dissipation of all previous contraction heat, the "unprotected" thermopile gave a much more rapid deflexion than the "protected" one, due to a warmer piece of muscle coming on to it in contraction, going off it in relaxation. For example, with a 0·3 sec. tetanus, 10 g. initial load, 27 g. afterload, the deflexions of Table III were obtained (reduced to maximum = 1000 in either case) after 15–20 min. rest. The difference between "protected" and "unprotected" is due to the resting heat.

When stimuli were applied in fairly rapid succession, e.g. at 1 min. intervals, the "protected" thermopile gave a series of almost identical

Table III—Comparison of Deflexions after Long Rest: "Protected" and "Unprotected" Thermopiles: 0·3 sec. Isotonic Tetanus

Time (sec.)	0	0·2	0·4	0·6	0·8	1·0
Deflexion: "Protected"	0	205	494	853	965	994
"Unprotected"	0	412	610	905	967	1000

records. The records, however, of the "unprotected" thermopile showed a large progressive change. The deflexions of Table IV were reduced to the same maximum, at 1·0 sec.; all except the first, however, showed two maxima, the earlier at 0·6 sec. or before. The records should all have been the same, as they were with the "protected" thermopile: in fact, they differed greatly from one another and from the records with the "protected" thermopile. An analysis of them would give an absurd result, much too large a positive heat at the start, then a considerable negative heat, then a positive heat later. This is in accordance with expectation. Even the absolute value for the total heat, if taken as given by the maximum deflexion, is considerably in error. All this shows the influence of the undissipated remainder of the heat of earlier contractions. Under isometric conditions this progressive alteration in a series made at short intervals did not occur.

Table IV—Deflexions from "Unprotected" Thermopile, 0·3 sec. Isotonic Tetani, in Fairly Rapid Succession

Time (sec.)	0	0·2	0·4	0·6	0·8	1·0
Deflexion: After long rest	0	412	610	905	967	1000
1 min. later	0	545	803	1023	987	1000
2 min. later	0	505	890	1085	985	1000
3 min. later	0	480	893	1108	997	1000
4 min. later			No record			
5 min. later	0	455	1030	1183	997	1000

With shorter tetani the difference was not so obvious to inspection, but immediately to measurement. For example, with 0·1 sec. isotonic tetanus, initial load 4 g., after-load 36 g., the "protected" thermopile gave its maximum deflexion at about 0·8 sec., the "unprotected" at about 0·3 sec. An analysis of the latter record would give a large negative heat in relaxation: a completely false result.

If, as has been customary hitherto, the whole thermopile, i.e. *CG*, had been used, the error in shortening experiments would have been "diluted" by adding the e.m.f. of the "protected" part to that of the "unprotected" part. The analysis would have been incorrect, but not obviously absurd: the total heat would have had an unknown error, probably not so large as altogether to invalidate the conclusions. It is not suggested that the results of all experiments hitherto made on the heat production of muscles allowed to shorten are wrong: it is certain, however, that they were affected by an error of unknown size, which may well have been large enough to obscure the finer details of the conclusions to be drawn from them. They ought all to be repeated by a method above reproach.

Fortunately such a method is at hand in the use of the "protected" thermopile. We have only to make sure that no part of the muscle can possibly come on to the thermopile which has not previously been subjected to identical conditions of heat loss. The simplest and safest way to do this is to continue the thermopile itself as a "dummy" below, and to a slight degree above, the part used to provide the deflexion. This is being done in the very thin new thermopile referred to in Part I. By its use it is hoped to reinvestigate the heat production of muscles allowed to shorten. Its thinness and quickness should render analysis easy and accurate, and the "protection" should make the deflexions truly represent the heat produced.

My thanks are due to Mr. P. E. Marrack of the Admiralty, for his help with the mathematics of Part I.

SUMMARY

A new method is described of analysing the time-course of the initial heat production of muscle. A very thin thermopile (45–60μ) is used with a heat capacity so small that the delay in taking up the temperature of the muscle can be neglected. The e.m.f. of the thermopile, and thus the temperature of the muscle, is obtained by a simple analysis (Appendix II) of the galvanometer record. No "heating control" is required except for the delayed heat.

A mathematical treatment of the heat flow from muscle to thermopile shows that the speed is adequate. A thinner thermopile would give little further advantage. The calculations are confirmed by direct measurement with thicker thermopiles.

The thin thermopile, of low thermal capacity and conductivity, avoids the necessity of "calibration" with a known amount of electrical energy: deflexions can be transformed directly into absolute units from the constants of the apparatus.

An error is discussed which must have affected, to a greater or less extent, all experiments hitherto made on the heat production of muscles allowed to shorten. The part of a muscle off a thermopile is less effectively cooled than the part on it, due to heat loss by conduction in the wires in the latter case, so that—owing to the resting heat, the recovery heat and the undissipated remainder of the initial heat of previous contractions—it is warmer. The error is caused by the warmer part coming on to the thermopile during contraction. It may be very serious. It can, however, be avoided altogether by using a "protected" thermopile, i.e. one with a "dummy" extension of identical thermal conductivity.

The method using a thin thermopile does not require that a muscle should be of uniform cross-section.

A moving coil galvanometer of short period is described which is sufficiently sensitive for most myothermic experiments.

APPENDIX I—A GALVANOMETER OF SHORT PERIOD FOR MYOTHERMIC EXPERIMENTS

BY A. C. DOWNING

The galvanometer is of the same general design as the Moll "Micro-galvanometer" manufactured by Messrs. Kipp of Delft, but owing to a lighter mirror and coil is more sensitive for a given period. The bifilar suspension is of gold alloy, to keep the resistance low. Non-magnetic silver wire is used for the coil.

The model employed in Professor Hill's recent experiments was as follows:

Coil measurement	...	22 × 1·5 mm.
Number of turns	...	35
Coil resistance	...	18 ohms

The coil is wound about a glass tube of about 0·2 mm. diameter, great care being taken to keep it symmetrical. To connect the ends of the coil and also the suspension, small pieces ("tags") of copper or silver wire are fixed in the ends of the tube, and the coil and suspensions are soldered to these. The suspensions are connected as near to the centres as possible, so that the final balancing (which must be carefully carried out) can be done by slight bending of the tags.

The suspension consists of two pieces of 10μ, 18 ct. gold wire, about 4 cm. in all. The total resistance is about 40 ohms. The gold naturally shows a certain amount of hysteresis, but if readings are taken in one direction only this disappears after the initial deflexion. For critical damping with thermopiles of rather high resistance the magnetic field must be as strong as possible, and a cobalt steel magnet is used. With the number of turns given above and a period of 0·2 sec., the galvanometer can be damped, using a magnetic shunt, with external resistances from 0–90 ohms. With its internal resistance of 60 ohms and a thermopile of 90 ohms, critically damped, its sensitivity is as follows:

Ampere sensitivity:
1 mm. at $2\frac{1}{2}$ m. distance = $4{\cdot}5 \times 10^{-9}$ amp.

Volt sensitivity:
1 mm. at $2\frac{1}{2}$ m. distance = $6{\cdot}7 \times 10^{-7}$ V.

A thermopile with 70 couples of constantan-manganin gives 2800 μV ($2{\cdot}8 \times 10^{-3}$ V) per 1° C., i.e. 4·2 mm. deflexion for 0·001° C. With photographic records readable to 0·1 mm., this is sufficiently sensitive for most myothermic experiments. For single twitches rather more sensitivity is required, and a similar and rather better galvanometer, with somewhat longer period, is being constructed.

APPENDIX II—THE ANALYSIS OF A GALVANOMETER RECORD TO DETERMINE THE CURRENT AS A FUNCTION OF THE TIME

For simplicity assume that the galvanometer is critically damped and that its period on open circuit is T. Then its deflexion at time t after putting a constant current into it is

$$y = 1000(1 - e^{-\alpha t} - \alpha t e^{-\alpha t}),$$

where $\alpha = 2\pi/T$ and 1000 represents the final steady deflexion. A galvanometer can be very accurately adjusted to critical damping and its period can be very accurately measured. A large-scale diagram has been constructed in which y, calculated from the above formula, is plotted against αt. From this, y can be read off at any required values of t, for any value of α.

Example of Analysis—A dead muscle on a thermopile was "instantaneously" heated by a condenser discharge, 0·7 μF, 315 V. The muscle being of about 5000 ohms the heating occurred at a decreasing exponential rate with time constant 1·75 msec. It was desired to analyse the time-course of the e.m.f. of the thermocouple, i.e. to determine the rise of temperature of its hot junctions, in units of 0·04 sec.

The period being $T = 0{\cdot}2205$ sec., $\alpha = 2\pi/T = 28{\cdot}5$. The calculated deflexion of the galvanometer to a constant current, read off from the chart at intervals of 0·04 sec., is given in Table V.

TABLE V—CALCULATED DEFLEXION OF GALVANOMETER TO CONSTANT CURRENT, FOR $T = 0{\cdot}2205$ SEC.

Time (units of 0·04 sec.)	0	1	2	3	4	5	6	7	8	9
Deflexion	0	315	664	855	942	977	992	997	999	1000

The observed deflexion when the muscle was "instantaneously" warmed, and the analysis obtained by subtracting successive fractions of the set of numbers in Table V, are given in Table VI. This particular experiment is that referred to in connexion with fig. 2 above. The thermopile was a very thick one and its temperature rise was slow: the deflexion, however, was quicker than that from a thin thermopile on which is a muscle contracting at 0° C.

TABLE VI—ANALYSIS OF GALVANOMETER DEFLEXION TO GIVE THE CURRENT

Time (units of 0·04 sec.)	0	1	2	3	4	5	6	7	8	9	10
Deflexion to be analysed	0	4	32	102	193	285	366	438	499	549	595
Subtract 0·013 at 0		0	23	91	181	272	353	425	486	536	582
„ 0·073 at 1			0	43	119	203	282	353	413	463	509
„ 0·137 at 2				0	28	86	153	219	277	327	372
„ 0·089 at 3					0	27	77	135	190	239	283
„ 0·085 at 4						0	20	62	110	156	199
„ 0·065 at 5							0	19	55	95	136
„ 0·060 at 6								0	15	45	79
„ 0·048 at 7									0	12	38
„ 0·040 at 8										0	11
„ 0·036 at 9											0
Current in interval	0·013	0·086	0·223	0·312	0·397	0·462	0·522	0·570	0·610	0·646	—

The mean current in each interval was obtained by adding the quantities given in the left-hand column, up to and including the interval considered.

The "remainders" in Table VI are all zero, and the analysis allows no discretion. Fig. 2 shows the result to be reasonably smooth. Thus a galvanometer of period 0·22 sec. is sufficiently rapid to allow a determinate analysis of the current to be made in units of 0·04 sec. This is about the shortest interval for which even the quickest thermopile would yield significant results.

Choice of Galvanometer—For analysing in units of 0·1 sec. we want the deflexion of the galvanometer 0·1 sec. after putting a constant current into it to be as great as possible. For a given galvanometer coil, adjusted to critical damping for a given external resistance by adjusting the magnetic field, the sensitivity is proportional to $T^{\frac{3}{2}}$ (see Hill 1934). For Table VII the deflexion is calculated at 0·1 sec. for a series of different periods of the same galvanometer coil. The greatest deflexion at 0·1 sec. is for a period of about 1·0 sec. Since, however, it is required to make photographic records on a drum of limited size, it is better to use a quicker and so less sensitive galvanometer, so long as the deflexion at 0·1 sec. is not too much reduced. A good compromise would be to use a galvanometer of period about $\frac{1}{3}$ sec., which would give good deflexions at 0·1 sec. and not too large deflexions later.

TABLE VII—DEFLEXIONS (TO CONSTANT CURRENT) AT 0·1 SEC. FOR A SERIES OF GALVANOMETERS, ALL WITH THE SAME COIL BUT OF DIFFERENT PERIODS, SET TO CRITICAL DAMPING BY ADJUSTING THE MAGNETIC FIELD, FOR THE SAME EXTERNAL RESISTANCE

Complete period (sec.)	2	1	0·5	0·4	0·333	0·25	0·15
αt at $\frac{1}{10}$ sec.	0·314	0·628	1·256	1·570	1·884	2·513	4·19
Deflexion at $\frac{1}{10}$ sec. (fraction of maximum)	0·040	0·131	0·358	0·465	0·561	0·716	0·921
Deflexion at $\frac{1}{10}$ sec. (absolute)	905	1054	985	940	863	716	429

APPENDIX III—THE CONSTRUCTION OF THIN THERMOPILES FOR MYOTHERMIC EXPERIMENTS

BY A. C. DOWNING

The wire used in the elements of these thermopiles is 42 s.w.g. (0·10 mm.) constantan-manganin. Constantan-iron provides a higher thermo-e.m.f. and a lower electrical resistance, but owing to the higher thermal conductivity of the iron the heat loss is greater. The junctions are made by hard soldering the wires end to end. They are then thoroughly cleaned and any excess of solder is removed by a small carborundum stick, after which they are rolled out with one rolling on jeweller's rollers. After many trials 15μ was found to be the minimum thickness to which 42 s.w.g. wire could be rolled and still give a straight couple. Straightness is important since, for sensitivity, the maximum number of couples in a given space is aimed at, and if the wires are badly bent the final result is disappointing.

A thin piece of mica is obtained, also approximately 15μ thick, by flaking from a thick piece of the best quality sheet. Less than 15μ does not give sufficient rigidity for mounting the wires. The mica is moistened with the smallest possible amount of bakelite varnish, and the couples are laid on it, each being carefully fixed in position under a low power binocular microscope. Soft solder is used for the cold junctions, which are "loaded" with a small piece of rolled copper or manganin (38 s.w.g.) to hinder their warming up by conduction. The best way to separate and solder these junctions is to thread a thin piece of mica through alternate pairs. In order further to hinder conduction to the cold junctions the distance from hot to cold on either side is 10 mm.: a shorter distance diminishes the electrical resistance, but allows the cold junctions to warm too quickly.

Bakelite varnish is now applied to the exposed surface of the thermopile and another 15μ piece of mica is laid on it so that the wires are sandwiched between them. This piece of mica is cut so that the upper surface of the cold junctions is left exposed. The element is left in the air to dry for 24 hr. To ensure perfect flatness and to make the element as thin as possible it is now pressed by a miniature vice between two plane parallel blocks: this squeezes out any excess of varnish and so makes good contact of the wires with the mica. The element, still held in the vice, is then baked at a temperature of 120° C.

The element is now ready for mounting in its metal frame, which has already had many applications of bakelite varnish and has been heat-treated several times at 120° C. Plenty of varnish is spread on the frame, and the element is simply laid in position in this. After drying in air for several days the whole is heat-treated as before. Perfect alignment of the element is important, and if the instrument is baked too early, while much solvent is still present, the latter may bubble and force the element away from the frame.

The galvanometer leads, which have been run down and fixed by bakelite varnish to the side of the frame, may now be connected. The upper surface of the cold junctions is still exposed, and is insulated and covered after the connexions have been made. Baking after this final insulation is not advised, for fear of bubbling, and the varnish should be allowed to air-dry for several days. The thinnest possible film of varnish rubbed over and into the mica seems to improve the insulation, and after a few weeks of drying the resistance between the thermopile and Ringer's solution in which it is soaked may be hundreds of megohms.

REFERENCES

Azuma, R. 1924 *Proc. Roy. Soc.* B, **96**, 338–48.
Beresina, M. 1932 *J. Physiol.* **76**, 170–80.
Cattell, McK. 1932 *J. Physiol.* **75**, 264–71.
v. Euler, U. S. 1935 *J. Physiol.* **84**, 1–14.
Feng, T. P. 1932*a* *J. Physiol.* **74**, 441–54.
— 1932*b* *J. Physiol.* **74**, 455–70.
Fenn, W. O. 1923 *J. Physiol.* **58**, 175–203.
— 1924 *J. Physiol.* **58**, 373–95.
Hartree, W. 1925 *J. Physiol.* **60**, 269–75.
— 1931 *J. Physiol.* **72**, 1–26.
— 1933 *J. Physiol.* **79**, 492–9.
Hartree, W. and Hill, A. V. 1928*a* *Proc. Roy. Soc.* B, **103**, 234–51.
— — 1928*b* *Proc. Roy. Soc.* B, **104**, 1–27.
Hill, A. V. 1913 *J. Physiol.* **46**, 435–69.
— 1925 *J. Physiol.* **60**, 237–63.
— 1928 *Proc. Roy. Soc.* B, **103**, 138–62.
— 1930 *Proc. Roy. Soc.* B, **107**, 115–31.
— 1931*a* *Proc. Roy. Soc.* B, **109**, 267–303.
— 1931*b* "Adventures in Biophysics." Univ. of Pennsylvania Press.
— 1934 *J. Sci. Instrum.* **11**, 309–13.
Solandt, D. Y. 1936 *J. Physiol.* **86**, 162–70.
Wyman, J. 1926 *J. Physiol.* **61**, 337–52.

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Native and Denatured Muscle Proteins

BY E. C. BATE SMITH

From the Low Temperature Research Station, Cambridge

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Meyer and Weber (1933) have recently proposed the addition of a third protein to the accepted constituents of muscle plasma, von Fürth's myosin and myogen. The evidence for the existence of "Globulin *X*", as it is tentatively named, is based on the examination of the precipitates formed during the dialysis of muscle press-juice and salt extracts. The salt-soluble fraction of the precipitate from muscle juice differs little from myosin in its isoelectric point, but it differs widely in its solubility in salt solutions and in the low viscosity of such solutions and their freedom from stream anisotropy.

The clean separation of the muscle protein fractions is rendered difficult by the readiness with which certain of them undergo denaturation. Not only does the formation of denaturation products multiply the number of individual substances present in press-juice and extracts, but the products themselves, although insoluble, form refractory suspensions which interfere with centrifugation and filtration. The object of the present work has been twofold: firstly, to ensure that denaturation products or complexes formed between native and denatured protein have not been mistaken for new native proteins; and secondly, to investigate the possibility of preparing cleanly separated fractions for the purpose of chemical and physico-chemical studies. The assurance of a pure preparation is particularly desirable in the case of myosin, which is at the present time receiving much attention in view of its importance in the physiology of muscular contraction.

As a result of this investigation, the separate existence of globulin *X* is confirmed. In addition, evidence of the existence of a fourth native protein has been found and it has been isolated in a denatured form. This protein has the properties of an albumin and will be referred to as myoalbumin. It differs from myogen in its low isoelectric point, in its solubility at high salt concentrations, and in its behaviour when treated with dilute acids. With regard to the clean separation of the various fractions, there is every reason to believe that myosin as ordinarily prepared consists of a single native protein species. Myogen cannot be separated from myoalbumin in the native state, but the denatured proteins can be fairly readily separated. Globulin *X* is the most difficult individual fraction to prepare owing to the tendency of denatured proteins to disperse in its solutions. The most important differences between the four fractions are summarized in a table on p. 149.

For the purpose of differentiation the following properties were taken into account:

(1) Isoelectric point.
(2) Solubility in water.
(3) Solubility in aqueous solutions of salts: viscosity and stream anisotropy of solutions.
(4) Solubility in dilute acids; *p*H at which reprecipitation occurs; solubility of reprecipitated protein.
(5) Character of precipitates.

Isoelectric points were determined by the cataphoretic method, using 0·01 M potassium phthalate or phosphate buffers in the apparatus described by Dummett and Bowden (1933). Most of the substances examined were insoluble in the buffer solution, and were prepared for cataphoresis by grinding in a mortar with a little of the buffer solution. Soluble fractions were either heat-coagulated and similarly treated, or the protein was adsorbed on quartz particles of suitable dimensions (cf. Abramson 1929). Observations were made at several different levels in the capillary tube; it was usually not possible to determine the absolute velocity owing to the rapid rate of fall of the particles. In no case, however, was there observed a difference in direction of movement at different levels. By qualitative observation it was usually possible to determine the isoelectric point to within ± 0·2 *p*H, and, in the best circumstances, to within ± 0·1 *p*H.

PREPARATION OF MYOGEN AND DETECTION OF MYOALBUMIN

The musculature of a rabbit was perfused with ice-cold 1 % sodium chloride solution via the thoracic aorta until the fluid issuing from an incision in the right auricle was almost colourless. The longissimus dorsi and hind-limb muscles were cut out, minced, and ground with washed sand. After standing for 1 hr. at room temperature the mass was pressed through well-washed filter-cloth in a strong press and the juice centrifuged and filtered. It was then dialysed to a conductivity equal to that of a 0·0005 M sodium chloride solution. During the dialysis a bulky precipitate settled out. This was centrifuged off. The clear supernatant liquid so obtained should consist of a solution of myogen free from myosin and other globulins, but containing small amounts of myoglobin and metamyoglobin and any unidentified water-soluble protein.

Denaturation of the Albumins

Dialysed muscle serum has a *p*H between 5·8 and 6·2. When allowed to stand either at room temperature or at zero a precipitate slowly forms. As Finn (1932) has shown, the formation of this precipitate is accelerated by the addition of salt, and still more by the further addition of acid. Addition of acid in the absence of salt causes no precipitation, but on neutralizing a precipitate is immediately formed indicating that denaturation has taken place without flocculation. The precipitate has an isoelectric point at *p*H 6·3–6·7 and is largely if not entirely denatured myogen (cf. Weber). It is almost completely insoluble between *p*H 5·8 and 7·5, and if salt is present the zone of insolubility widens until, when more than 0·5 M potassium chloride is present, flocculation occurs on the acid side even at *p*H 2.

When an equal volume of N/100 hydrochloric acid is added to the dialysed serum and the solution is neutralized, most of the protein is precipitated in the denatured form. When the solution in acid, which has a *p*H between 3 and 4, is kept at room temperature, or if it is heated to 100°, further amounts of protein are denatured (still without appreciable flocculation), but a fairly constant residue of 10–15 % of the protein remains in solution after neutralization. This fraction has an isoelectric point at 4·4–5·2, and when it is kept at any *p*H between 2 and 7 a precipitate is very slowly formed, part or all of which is insoluble in acid. This insoluble fraction is colourless and has an isoelectric point at 3·0–3·5, widely different from that of myogen, myosin (*p*H 5·5), globulin *X* (5·2), and the blood-serum proteins (4·7–5·5). None of these fractions when similarly kept forms a precipitate insoluble in dilute acid. The precipitate consists, therefore, of the denatured form of a hitherto undescribed fraction of the muscle proteins, the myoalbumin previously referred to.

The denatured myogen which separates on neutralization is practically free from myoalbumin, since no precipitate forms when it is redissolved in acid and kept for many days at *p*H 2–4. The isoelectric point of this myogen is *p*H 6·5–6·7. The slightly lower values which have previously been recorded and which are obtained when dialysed muscle juice is examined without further fractionation (Weber 1925) are probably due to admixture with myoalbumin.

It has not been found possible to separate myoalbumin in the native state completely from myogen by treatment with acid,* although preparations richer in myoalbumin than the original muscle serum can be made. Firstly, by fractional precipitation with ammonium sulphate; the fraction separating at slightly more than half saturation gave 11 % of soluble residue after acidification to *p*H 2 and neutralization; that separating at complete saturation (actually the whole of the remaining protein) gave 22·5 % of residual soluble protein. The isoelectric points of these residues (4·5–5·0) indicated that they still consisted of mixtures of myogen and myoalbumin, but the high content of undenatured protein in the second fraction must be taken to indicate a higher myoalbumin content. By fractional heat coagulation of the residual soluble protein from another preparation it was possible to show that myoalbumin has a higher heat-coagulation temperature than myogen. The solution was placed in a test tube in a water-bath, the temperature of which was slowly raised. Heat coagulation began at 48°, and at 55° a marked coagulum had appeared. This was filtered, and the filtrate was heated successively to 62·5, 72, 80 and 90°. At each stage the precipitate was collected and washed. The first sample was isoelectric at *p*H 5·1, that separating between 72 and 80°, at 4·7, and that between 80 and 90°, at 4·55. None of these fractions was either pure myogen or myoalbumin, but those coagulating at the higher temperatures were undoubtedly richer in myoalbumin. Weber (1927) had rightly suspected the existence of this second albumin from the fact that a small amount of protein remains uncoagulated when muscle juice is heated to 70°.

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* As Anson and Mirsky have shown, there is in such circumstances an equilibrium between native and denatured protein so that denaturation of myogen would not proceed to completion.

The amount of myoalbumin present in muscle juice is difficult to assess accurately, since it is impossible to determine how much myogen is associated with it in the soluble residual fraction. It can, however, scarcely amount to more than 10 % of the total albumin of the juice.

MUSCLE GLOBULINS

According to Edsall (1930) "muscle globulin" (myosin) is prepared by suspending the minced, perfused muscles in a buffered solution of potassium chloride, stirring, filtering, and diluting the clear sol with twenty times its volume of distilled water. The final concentration of salt is approximately 0·07 M with respect to potassium chloride. The precipitated globulin is centrifuged, and for purification is taken up in salt solution and reprecipitated either by salting out with concentrated potassium phosphate or by dilution. Edsall recommends that these processes be carried out alternately. The properties of myosin, so prepared, have been described by Weber (1925), Edsall and von Muralt (1930), Smith (1934*a*), Astbury and Dickinson (1935), etc. Briefly, it is characterized by an isoelectric point at about *p*H 5·5; between *p*H 5 and 8, in the absence of salt, it exists in the form of an opalescent gelatinous mass which is thixotropic, and its highly viscous solutions exhibit anomalous viscosity and strong stream anisotropy. It has well-defined solubility relations depending on salt and hydrogen-ion concentration. It is capable of being drawn into strong threads which give a characteristic X-ray diffraction pattern which changes on stretching in a manner similar to that of keratin.

Denaturation of Myosin

a—Effect of Treatment with Acid—When treated with dilute mineral acid, in the absence of salt, myosin dissolves at a *p*H slightly below its isoelectric point, and is scarcely changed in properties when the solution is neutralized. The reprecipitated protein disperses completely in salt solutions, the form of the solubility curve being identical with that of the untreated myosin. Although its solution in acid no longer shows stream anisotropy this property is recovered after neutralization and dissolution in salt.*

b—Effect of Temperature of Storage—When kept in the cold, myosin does not denature, but if the temperature is raised slightly above room temperature, the protein gradually loses its solubility in salt solutions. Fig. 1 shows the effect of keeping the salt-free protein for 24 hr. at 25 and 30°. (A small insoluble coagulum is also gradually formed in this temperature range.) At 40–45° the protein rapidly coagulates. The gradual shift of the solubility curve to the left probably indicates the existence of intermediate stages between the native and the heat coagulated protein.

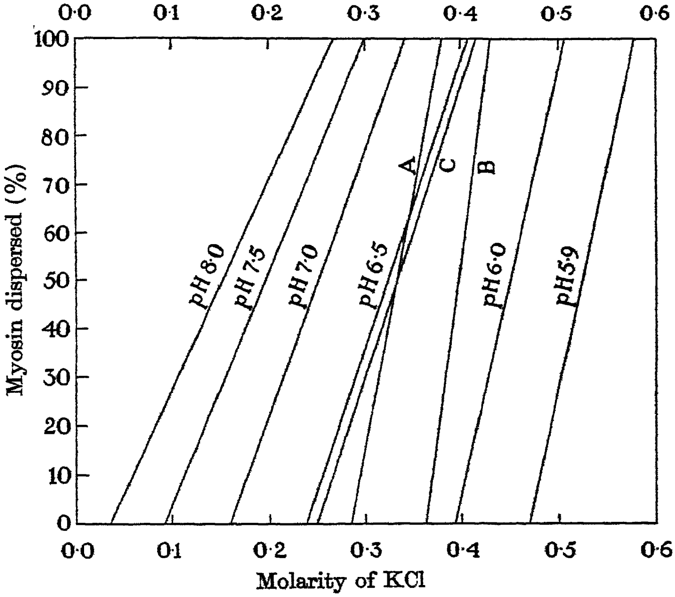

FIG. 1—Effect of keeping for 24 hr. at pH 6·7 (A) at 25° and (B) at 30° on the solubility curve of myosin. The "dispersing concentration" (compare Smith 1934*a*) is little changed in the former case, but is increased in the latter, and in each the threshold concentration is much increased. C shows the solubility curve of myosin from rigor muscle; it is identical with that from fresh muscle.

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* Strong stream anisotropy was observed in a preparation washed thoroughly free from salt, acidified to pH 3 with N/10 hydrochloric acid, neutralized with N/10 sodium hydroxide, and redissolved by addition of solid potassium chloride, although Muralt and Edsall report that this property is lost by treatment of myosin with acid.

Solubility of Myosin in Dilute Salt Solutions

The previous study of the solubility of myosin was concerned primarily with salt concentrations equal to or greater than that of its natural environment. The interest in the present work lies in its behaviour in dilute salt solutions such as diluted muscle extracts or dialysed press-juice.

The solubility curve of myosin with increasing salt concentration is characterized by two points of inflexion: that at which the solubility suddenly begins to rise with great rapidity, and that at which the whole of the myosin present is dispersed. Between these two points the relation between salt concentration and percentage dispersion is a straight line, which, if extrapolated, cuts the base line at a point corresponding to nil dispersion. The salt concentration at this point was termed the threshold concentration. At *p*H 6·0 the threshold for potassium chloride is 0·4 M, at 6·5, 0·25 M, and at 7·0, 1·7 M. For several-times precipitated myosin suspended in potassium chloride solutions this threshold has a real existence, but in certain circumstances the solubility curve departs from the straight line and curves away towards zero. One such circumstance is the presence in the myosin solution of inorganic phosphates, and the protein prepared by Edsall's method retains sufficient phosphate even after repeated washing with distilled water to allow appreciable dispersion in highly diluted salt solutions. If the phosphate is still further attenuated by redissolving the myosin in 10 % potassium chloride and reprecipitating, the protein is found to be completely insoluble below the threshold concentration of salt. The following experiments illustrate this effect of phosphate.

Twice-precipitated myosin, prepared as described above, was dissolved by addition of potassium chloride to 1·4 M concentration, the *p*H of the sol being 6·8. The sol was diluted with distilled water to 0·07 M potassium chloride and allowed to stand at 0° for 18 hr. The precipitated myosin was centrifuged off, and the supernatant liquid was treated with an equal volume of 10 % trichloracetic acid. No perceptible precipitate was formed; in other words, the recovery was 100 %. (In similar circumstances, once-precipitated myosin constantly gives a recovery of 88 %.)

The experiment was repeated with 1·6 M potassium phosphate at *p*H 6·45, recovery 80 %; with 1·4 M potassium phosphate at *p*H 6·2, recovery 87 %; and with 1·4 M potassium chloride containing 0·06 M potassium phosphate at *p*H 7·0, recovery 89 %. Hence, at *p*H 7·0 as little as 0·003 M phosphate is able to effect 11 % dispersion of myosin in presence of potassium chloride below the threshold concentration, and 0·07 M phosphate at as low a *p*H as 6·2 will effect 13 % dispersion. Until, therefore, the phosphate present in muscle extracts is removed by repeated precipitation, myosin cannot be completely removed from solution by dilution.

The globulin of Muscle Press-juice: Globulin X

Potassium (0·13 M), phosphate (0·065 M) and lactate (0·05–0·1 M) are the preponderating ions of muscle juice, which usually has a *p*H between 5·8 and 6·2. Although in this range of *p*H the threshold concentration for potassium chloride (and presumably also for potassium lactate) is 0·3–0·4 M it might be expected that the phosphate present would cause quite appreciable amounts of myosin to dissolve in the juice. When a typical sample of muscle juice is dialysed globulin amounting to about 10 % of the protein present is precipitated but most of this, as Weber and Meyer showed, differs in important respects from myosin.

The isoelectric point of the globulin is 5·0–5·2, not far removed from that of myosin which is at 5·3–5·5. Its solutions are less viscous, and show no stream anisotropy. When precipitated by dialysis, the globulin sediments rapidly, whereas myosin forms a slowly settling opalescent cloud; and when centrifuged it packs into a tight pad, whereas myosin cannot be closely packed but remains in the form of a loose gel-like suspension. If the *p*H is not above 6·4, globulin *X*, like myosin, can be precipitated by dilution of its sol in 10 % potassium chloride or 7 % lithium chloride. By twenty-fold dilution at *p*H 6·0–6·2 the recovery is 60–80 %, whilst that of once-precipitated myosin is about 90 %.

These differences might conceivably be the result of alteration of myosin during the long process of dialysis, or they might be due to the formation of an intimate mixture of myosin with denatured albumins. The distinction between this globulin and myosin is, however, conclusively proved by the difference in their behaviour when treated with dilute acid. Globulin *X* readily dissolves in acid and when neutralized to *p*H 5 it is reprecipitated, but unlike myosin the reprecipitated protein is completely insoluble in salt solutions, and is therefore denatured. The final proof that the globulin of muscle juice is almost entirely free from myosin is furnished by the following quantitative experiment based on this distinction between the two proteins.

The dialysis precipitate from muscle juice prepared as previously described was washed free from albumin with distilled water. It was then suspended in 7 % lithium chloride, centrifuged, and the supernatant liquid dialysed to 0·02 M salt. The precipitated globulin was washed, taken up in 0·1 N hydrochloric acid, allowed to stand overnight, neutralized to *p*H 5·3, centrifuged, and taken up in buffered 10 % potassium chloride at *p*H 7. Only 5 % of the protein passed into solution. This represents the limit of the myosin content of the globulin precipitate.

Denaturation on Keeping

Globulin *X* resembles myogen in the ease with which it is denatured by dilute acids; like myogen, it also denatures when allowed to stand in a neutral medium, either at room temperature or in the cold, and in these circumstances the denatured protein coagulates. A clear sol of globulin *X* can seldom be prepared, since part of the flocculated protein is peptised by the dissolved native protein, and cannot be separated by filtering or centrifuging. For this reason it is difficult to determine accurately either the rate of denaturation in different circumstances, or the solubility relations of globulin *X* in salt solutions. With regard to the latter, two observations have been made.

1—At the *p*H of muscle juice (5·8–6·2) globulin *X* is partially precipitated by twentyfold dilution of its solution in 1·6 M lithium chloride or 1·8 M potassium chloride, about 20–30 % of the protein remaining in solution.

2—At the *p*H of the Edsall extract of muscle (6·9–7·3) the protein is not precipitated by twentyfold dilution of its solution in 1·4 M potassium chloride. Unless, therefore, it forms an adsorption complex with myosin, the latter protein as normally prepared must be free from globulin *X*. In actual fact, no globulin *X* can be detected by the hydrochloric acid reaction either in the myosin as a whole or in any of the fractions obtained by fractionation with salt solutions. For instance, the 10–12 % of protein remaining in solution after dilution of a sol of once-precipitated myosin in potassium chloride-phosphate contains no detectable trace of globulin *X*. It must therefore be concluded that myosin as normally prepared is completely free from globulin *X*.

Quantitative aspects

1—*Distribution of the soluble proteins in muscle.*

A scheme for the approximate determination of the then recognized protein constituents of muscle was published some years ago (Smith 1934*b*). In so far as myosin is concerned, little revision of this scheme is necessary. The maximum yield of native protein is achieved by exhaustive extraction with 7 % lithium chloride solution. By twentyfold dilution of this extract, 88 ± 2 % of the myosin, free from other constituents, is precipitated.

It remains to determine the distribution of globulin *X* and albumins in the solution remaining after removal of this precipitate and the constitution of the acid-soluble residue of the intracellular proteins not extracted by lithium chloride.

a—Non-myosin fraction of lithium chloride extract—The supernatant solution and washings from the myosin precipitation were dialysed to 0·004 M salt concentration. No further precipitate formed when dialysis was continued. The soluble proteins remaining therefore represented the albumin fraction. The precipitated protein was washed on the centrifuge and was found to be soluble without residue in 10 % potassium chloride. It therefore contained no denatured myogen. After removal of salt by dialysis, the protein was treated with N/10 hydrochloric acid at room temperature for 3 hr. The solution was neutralized and treated with 10 % potassium chloride. The protein which dissolved—about 50 % of the whole—was roughly equal to the calculated remnant of myosin. The insoluble fraction therefore represented the globulin *X* present in the extract.

Further extraction of the muscle residue with hydrochloric acid indicated that the lithium chloride extract had contained 82·5 % of the total intracellular protein, and the above analysis yielded the following results (in percentage of total intracellular protein):

Myosin	63 %
Globulin *X*	9 %
Myogen and Myoalbumin ...	10 %

b—Residual intracellular fraction—The 17·5 % of protein remaining after exhaustive extraction with lithium chloride appears to consist mainly of denatured globulin *X*. The evidence for this rests on the following experiment.

Five-gram samples of muscle, after exhaustive extraction with lithium chloride solution, were washed twice with water and were then extracted several times with N/100 hydrochloric acid. When the hydrochloric acid extract was carefully neutralized, the whole of the protein was precipitated below *p*H 5·5, indicating that myogen, which remains in solution in these circumstances until at least *p*H 5·8 is reached, formed only a small part of the precipitate. The precipitate was suspended in 10 % potassium chloride at *p*H 7. It was found that 25 % of the protein dissolved. This represents approximately the myosin content of the residual protein, the remaining 75 % being, therefore, mainly globulin *X*.

Accepting this estimate of the composition of the residual fraction, the complete analysis of the soluble proteins would be as follows: myosin 67–68 %, globulin *X* 20–22 %, myogen and myoalbumin 10–12 %.

2—*Distribution of proteins in muscle press-juice.*

The press-juice from muscle normally contains from 1·0–1·3 % of protein N. When the juice is dialysed to 0·0004 M salt concentration a fairly constant fraction—about 20 %—of the nitrogen is precipitated, and of this again a fairly constant fraction—about half—is soluble in Edsall's solution and consists entirely of globulin *X*. The insoluble fraction is mainly denatured myogen and globulin *X*, and is usually almost completely soluble in N/100 hydrochloric acid. The small insoluble residue is denatured myoalbumin, isoelectric at *p*H < 4. The native myoalbumin constitutes 10 % of the total albumin. The distribution of these fractions in the juice is, therefore (as percentage of total protein):

Myogen	...	75–80 %
Myoalbumin	...	5–7 %
Globulin *X*	...	15–17 %
Myoglobin	...	Trace

In whole muscle the ratio of globulin *X* to myogen is at least 2 : 1. At the *p*H at which the juice is expressed—5·8–6·2—most of this globulin is therefore undissolved. It seems quite likely, however, that in living muscle at *p*H 7·3–7·5 the whole of the globulin *X* would be soluble in the juice, since *p*H has considerable effect on the solubility of this fraction in dilute salt solutions. Myogen, myoalbumin and *X* would then provide a serious medium—the sarcoplasm—surrounding the myosin fibrils (cf. Weber 1934). The absence of myosin from muscle press-juice suggests that the surfaces of the fibrils are differently constituted from the interior, since there is, in rigor juice, ample phosphate to disperse as much as 10 % of the myosin present were the two systems intimately in contact. Weber's picture of the myosin and myogen systems as anatomically distinct is, therefore, in all probability, correct.

At the *p*H at which the juice is normally expressed, the sarcoplasm would represent about 30 % of the weight of muscle. This value is calculated from the average myogen content of the juice and of the whole muscle.

Average coagulable N of juice = 1·25 %
Approximate myogen ,, = 1·0 %
Average soluble protein N of muscle = 2·9 %
Approximate myogen ,, = 0·3 %

Assuming the juice to contain the whole of the muscle myogen

$$\frac{\text{Weight of juice}}{\text{Weight of muscle}} = \frac{0{\cdot}3}{1{\cdot}0} = 30\ \%.$$

The relative volumes of sarcoplasm and fibrils would be expected to vary with variation in *p*H, so that this affords little indication of the conditions in living muscle. The probable tendency would be for the fibril to shrink as the reaction becomes more acid; it would therefore occupy a relatively greater volume in living muscle than in rigor muscle.

Denaturation in situ

The reaction and salt concentration of the sarcoplasm of muscle in rigor are favourable to the denaturation of myogen and globulin *X*, but, so long as the temperature is kept below about 15°, the denaturation of myosin should be extremely slow. In the literature, surprisingly high values are found for the loss of solubility of the proteins in the first few hours post mortem. As was pointed out in earlier papers, no evidence of such a rapid denaturation could be found, and considerable further work has failed to produce a single instance of so rapid a change.

To hinder bacterial decomposition, in recent experiments the minced muscle has been stored in an atmosphere containing 60–100 % of carbon dioxide. The presence of this gas is unlikely to have any further effect than its anti-bacterial action since the shift in *p*H of muscle juice due to saturation with carbon dioxide is only 0·1. The effect of periods of storage at various temperatures on the amount of protein extractable from rabbits' muscle with 7 % lithium chloride is illustrated in the following table:

Experiment	Temperature of storage ° C.	Duration	% decrease in soluble protein
1	0, 10, 20	24 hr.	nil
	33	24 „	30 %*
2	30·5	24 „	39 %
3–5	0	3 weeks	8, 10, 8 %

* Some bacterial decomposition took place in this instance.

These and similar results published elsewhere (Smith 1934*b*) show conclusively that when mammalian muscle passes into rigor mortis no decrease in the total protein extractable with 7 % LiCl solution takes place.

This conclusion is, however, difficult to reconcile with the recent work of Deuticke (1930), amply confirmed by Meyer and Weber (1933), and by Mirsky (1936). Deuticke found that the amount of protein dissolved from frogs' and rabbits' muscle in rigor by an aqueous solution containing

4·56 g. of potassium dihydrogen phosphate and 4·86 g. of disodium hydrogen phosphate in 100 c.c. was notably less than that dissolved from fresh muscle. In similar experiments of Meyer and Weber this decrease was shown to be due to a decrease of 72 % in the amount of myosin extracted. Mirsky found that whereas 67·6 % of the protein was dissolved from frog's muscles immediately after death, only 38·3 % was dissolved from the muscles of frogs injected with iodoacetic acid and kept at 8° C. overnight. There are certain differences in procedure which may serve as a basis for explanation of these apparently contradictory results.

1—*Conditions of extraction*—Deuticke (1930) and Hensay (1930) make it quite clear that the effect could only be brought out by the use of a limited type of extractant solution. If, for instance, sodium acetate buffers were used, more protein dissolved from stored muscle than from fresh muscle. The effect diminished also if less concentrated phosphate solutions, or more acid or more alkaline solutions, were used. Further, the extraction was not exhaustive; the finely divided muscle was suspended in a single large volume of solution, and allowed to stand overnight with occasional mixing. The results quoted above (p. 147) were obtained by extracting 6–9 times, giving 95–98 % of the total extractable protein.

2—Mirsky, who used 10 % KCl-phosphate (cf. p. 140) as an extractant, induced rigor by injection of sodium iodoacetate.

Mechanism of Denaturation and Coagulation

In common with the denaturation produced by other agencies, Anson and Mirsky have shown that denaturation with acid usually results in the formation of active SH groups. The denaturation of myogen in muscle press-juice is probably a form of acid denaturation since it is rapidly accelerated by increase in, and is retarded by decrease in, hydrogen-ion concentration (Finn 1932). If myosin were similarly denatured in rigor muscle in which a *p*H as low as 5·5 may be reached, it would be expected that active SH groups would appear in proportion to the amount of insoluble proteins formed. Mirsky, however, finds no detectable increase in the number of active SH groups in rigor, as compared with fresh muscle, and concludes that the coagulation which occurs is of a type peculiar to myosin and allied to that brought about by dehydration.

In the light of the experiments described above, it seems, however, more reasonable to conclude that this is confirmatory evidence that myosin is not denatured or coagulated in muscle as it passes into rigor. The evidence of change in "solubility" is, actually, evidence only of a change in the accessibility of myosin to the extracting salt solution. It has been suggested in an earlier paper (Smith 1934*b*) that the efficiency of an extractant is determined not only by its ability to dissolve myosin, but also by its effect on the membranes supporting and more or less enclosing the fibrils. No investigator has yet succeeded in extracting the whole of the soluble protein from muscle with an aqueous solution of a neutral salt, although this can readily be done with dilute acid. It is significant that, although myosin is quite appreciably soluble in aqueous solutions of similar constitution to muscle serum, scarcely any trace of myosin can be found in the juice produced by simple expression. The only reasonable conclusion is that the myosin fibrils are surrounded by membranes (or effective barriers) of insoluble material, and that it is the permeability of these membranes with respect to a given extractant which primarily determines the extent to which protein dissolves. It follows that the differences observed between fresh and rigor muscle are not necessarily due to denaturation and coagulation of the contents of the fibrils but are, more probably, due to changes in the membranes which must be supposed to surround the fibrils.

Summary

1—The existence of globulin *X*, first isolated by Meyer and Weber, is confirmed, and some of the properties of a new fraction, myoalbumin, are described.

2—The four soluble proteins are differentiated by their isoelectric points, by their solubility in water and aqueous salt solutions and by their behaviour when rapidly acidified with dilute mineral acid and neutralized. The most outstanding differences are summarized in the following table:

		Solubility of protein			
		Before treatment		After treatment with acid	
	Isoelectric point	In water	In salt solutions	In water	In salt solutions
Myogen	6·5–6·7	+	+	0	0
Myoalbumin	3·0–3·5	+	+	+	+
Myosin	5·5	0	+	0	+
Globulin *X*	5·2	0	+	0	0

3—Myoalbumin slowly denatures at all values of pH between 2 and 7, the product being insoluble in dilute acid (distinction from myogen).

4—Myosin in complete absence of salt is unaffected by dilute acid. It will even regain the property of double refraction of flow when neutralized and redissolved in a salt solution.

5—No appreciable change in protein solubility occurs when muscle undergoes rigor mortis. The contradictory evidence on this point is discussed.

References

Abramson 1929 *J. Gen. Physiol.* **13**, 169.
Astbury and Dickinson 1935 *Nature, Lond.*, **135**, 95, 765.
Deuticke 1930 *Pflüg. Arch. ges. Physiol.* **224**, 1.
Dummett and Bowden 1933 *Proc. Roy. Soc.* A, **142**, 382.
Edsall 1930 *J. Biol. Chem.* **89**, 289.
Edsall and von Muralt 1930 *J. Biol. Chem.* **89**, 315, 351.
Finn 1932 *Proc. Roy. Soc.* B, **111**, 396.
Hensay 1930 *Pflüg. Arch. ges. Physiol.* **224**, 44.
Meyer and Weber 1933 *Biochem. Z.* **266**, 137.
Mirsky 1936 *J. Gen. Physiol.* **19**, 571.
— 1937 *J. Gen. Physiol.* **20**, 455, 461.
Smith 1934*a* *Proc. Roy. Soc.* B, **114**, 494.
— 1934*b* *J. Soc. Chem. Ind., Lond.*, **103**, 351 T.
Weber 1925 *Biochem. Z.* **158**, 443.
— 1927 *Biochem. Z.* **189**, 407.
— 1934 *Pflüg. Arch. ges. Physiol.* **235**, 205.

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The Menstrual Cycle of the Primates

XII—The Interaction of Ovarian Hormones in the Cycle

By S. Zuckerman

From the Department of Human Anatomy, Oxford

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[Plates 7, 8]

The following study was undertaken for the purpose of throwing light on the endocrine mechanisms that operate in menstrual cycles in which ovulation occurs, and in which the endometrium is consequently influenced by both the follicular and luteal hormones of the ovary. The immediate object of the investigation was to define experimental conditions that would conform temporally to the phases of the normal cycle, and under which uterine bleeding would occur at approximately 4-weekly intervals in ovariectomized rhesus monkeys treated with both oestrin and progestin. The information obtained in this study is complementary to observations that deal with the mechanism of anovular cycles (Zuckerman 1937*a*, *b*), and helps to provide a basis for the full interpretation of the menstrual cycle.

MATERIAL AND METHODS

Details about the spayed rhesus monkeys (*Macaca mulatta*) used in this study are given in a previous paper (Zuckerman 1937*a*), where the reactions of the animals to varied conditions of oestrogenic stimulation are described.

Injections were given daily and intramuscularly. The oestrogenic substance used was an oil solution of crystalline oestrone. The luteal hormone employed was either a commercial extract of corpora lutea (progestin), assayed in rabbit units, or a solution of crystalline progesterone in oil. The progestin was obtained from British Drug Houses Ltd., whose rabbit unit is approximately equivalent to 0·60 mg. free progesterone; from the Leo Company, whose unit is equivalent to 1·0 mg. of free progesterone; and from Organon Ltd., whose unit is also approximately equivalent to 1·0 mg. of free progesterone.

EXPERIMENTAL

(*a*) *The Effect of Progestin administered alone*

A spayed monkey (186·1) was given 2 R.U. progestin (= 2 mg. progesterone) daily for 14 days. Uterine bleeding did not occur either during or after this course of injections, a fact which agrees with the view that progestin has little or no effect on an endometrium that has not been previously acted on by oestrin (see Hisaw and Leonard 1930). Hisaw (1935) reports that oestrin-free progestin will cause slight growth in both the stromal and epithelial tissues of the endometrium of castrated rhesus monkeys, but he does not record the occurrence of uterine bleeding either during or after such treatment.

Bleeding will occur if a single dose of oestrone is given during a course of progestin injections. Thus a second and similar series of progestin injections was given to the same monkey referred to above (186·2). On the sixth day of the experiment the animal was also given 2000 I.U. of eostrone. Uterine bleeding began 4 days after the last injection of progestin, and lasted 3 days.

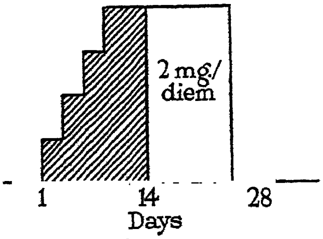

FIG. 1

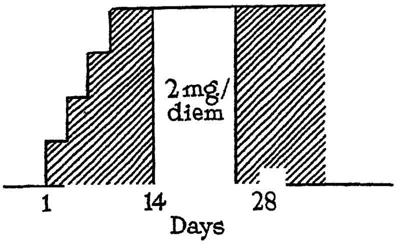

FIG. 2

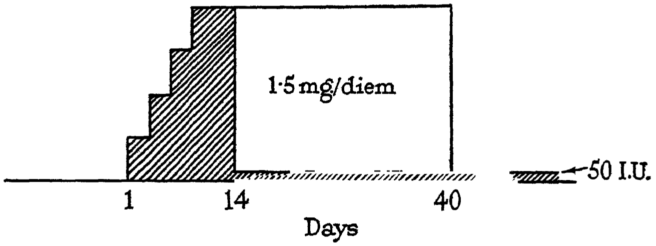

FIG. 11

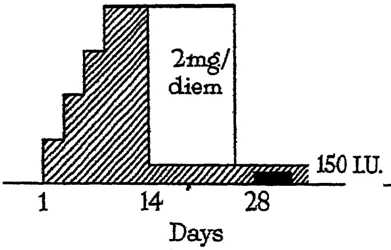

FIG. 3

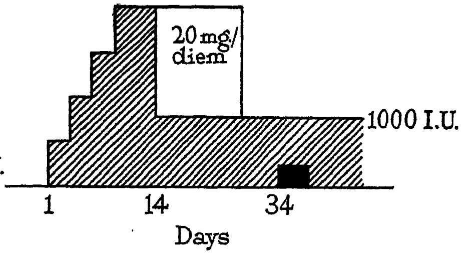

FIG. 4

Explanation of figs. 1, 2, 3, 4 *and* 11

The hatched and the unshaded areas indicate the duration and degree of oestrogenic and luteal stimulation respectively. The small black rectangular areas indicate phases of uterine bleeding. The diagrams are not drawn to scale. The maximum daily dose of oestrone in the first 14 days is in all cases to be taken as 3000 I.U.

FIG. 1—The uterine bleeding that normally occurs about a week after the cessation of injections of oestrin can be inhibited by the administration of progestin. Bleeding begins a few days after the progestin treatment is stopped.

FIG. 2—This bleeding is not inhibited if injections of oestrone are restarted after the end of the progestin treatment.

FIG. 3—The administration of progestin for periods of from 10 to 12 days during the second phase of an "artificial cycle", when oestrogenic stimulation is at a threshold level, neither hastens nor significantly retards the time of uterine bleeding.

FIG. 4—Uterine bleeding will occur in such experiments when the level of oestrogenic stimulation is well above the threshold value, provided that the amount (by weight) of progesterone given is about 100 or more times that of the oestrone administered during the same period.

FIG. 11—The continued administration of a sufficient amount of progestin will inhibit the uterine bleeding that would normally occur in the second phase of an "artificial cycle" in which an animal is being injected daily with a subthreshold dose of oestrin.

(*b*) *The Inhibition of Oestrin-Withdrawal Bleeding by Progestin*

It has been found that the uterine bleeding which normally follows the cessation of a series of oestrin injections can be inhibited by the administration of progestin; the bleeding does not occur until the progestin injections are stopped (fig. 1; see Smith and Engle 1932; Hisaw 1935; Engle, Smith and Shelesnyak 1935). On the other hand, the bleeding that occurs after the progestin injections are stopped cannot be inhibited by the further administration of oestrone. Thus a castrated monkey (70·7) was given oestrone in increasing amounts up to 2000 I.U. daily for 14 days (total amount injected = 2·05 mg.). From the 15th to 26th days inclusive it was given 2 R.U. progestin (= 2 mg. progesterone) daily. From the 27th day onwards it was given 500 I.U. of oestrone daily. Bleeding set in on the 29th day of the experiment, in spite of the restarting of oestrone injections (fig. 2). Similar results have been obtained both by Engle and others (1935) and by Hisaw (1935), and they suggest that once the stimulus that has produced progestational changes in the uterus is withdrawn, oestrone cannot prevent the consequent usual breakdown of the endometrium.

(*c*) *The Simultaneous Administration of Oestrin and Progestin*

The observations reported both in the preceding and in the first part of this series of papers (Zuckerman 1930, 1937*b*) show that the intensity of oestrogenic stimulation diminishes abruptly about the middle of the menstrual cycle, and that it falls to some point within a low and fairly fixed threshold range, the fall coinciding with ovulation and the end of the follicular phase. Luteal stimulation of the endometrium is thus normally confined to the post-ovulation phase (second half) of the cycle, and is presumably superimposed on a low level of oestrogenic stimulation. The following experiments were devised to throw light on the nature of the interaction of oestrogenic and luteal hormones in this phase of the cycle.

Ovariectomized rhesus monkeys were first injected daily for 14 days with increasing amounts of oestrone, the maximum dose given varying in different experiments from 1000 to 3000 I.U. daily. The first part of the experiment thus simulated the follicular phase of normal menstrual cycles. From the 15th day to the end of each experiment both oestrin and progestin were given daily in various combinations. Experiments ended when uterine bleeding took place. If no bleeding occurred during the course of injections, treatment was stopped on the 41st day and the animals were not injected again until after the occurrence of post-injection bleeding. Such experiments are indicated in Table I by the figures 40..., or 40 + 5 or 6, depending on the number of days intervening between the cessation of injections and the onset of uterine bleeding. Details of ten experiments are given in Table I.* In order to interpret them, it is necessary to compare them with parallel experiments in which no progestin was given, but in which the daily dosage of oestrone varied in the same way. The results of such control experiments are given in the last column of the table. Full details of these control experiments are given in the preceding part of this series of papers.

It will be seen that the administration of progestin did not materially affect the time of uterine bleeding in Exps. 1 and 2. Bleeding in both experiments presumably resulted from the cessation of luteal stimulation as well as from the low level of oestrogenic stimulation (fig. 3). Moreover, it is conceivable that the effective value of both hormones was reduced by their simultaneous administration and consequent antagonistic interaction (Hisaw and Leonard 1930; Leonard, Hisaw and Fevold 1932; and see below). In Exp. 3, on the other hand, an animal bled after the cessation of progestin treatment in spite of the fact that it was still being injected daily with an amount of oestrone which in a previous experiment had maintained the endometrium in a phase of growth. A similar result is demonstrated more conclusively by Exps. 4, 5 and 8. In no. 4 a monkey bled while it was being injected daily with 300 I.U. of oestrone, and after 12 days' previous treatment during which this amount of oestrone was given daily in conjunction with 5 mg. of progesterone. This animal did not bleed in a control experiment in which corresponding amounts of oestrone had been given alone. In no. 5, a monkey bled during the course of injections of 500 I.U. of oestrone daily, and after a period of 12 days in which this amount of oestrone was given daily in conjunction with 10 mg. of progesterone. A daily dose of 500 I.U. of oestrone is well above the oestrone-threshold for uterine bleeding in rhesus monkeys. Moreover, as Exps. 6 and 7 show, uterine bleeding will not occur in similar experiments in which 500 I.U. of oestrone are given daily if the ratio of progestin to oestrin during the period in which the two hormones are given together is too low. Exp. 8 is even more conclusive than those which precede it, for in this experiment an animal bled while being injected daily with 1000 I.U. of oestrone, after a 12-day period in which the same amount of oestrone was given daily in conjunction with 20 mg. of progesterone (fig. 4).

The first possibility that arises in the interpretation of these results is that the effective value of oestrin is considerably diminished when it is given in combination with progestin, and that the uterine bleeding which

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* Preliminary notes have been published about some of these experiments (Zuckerman 1936, 1937c).

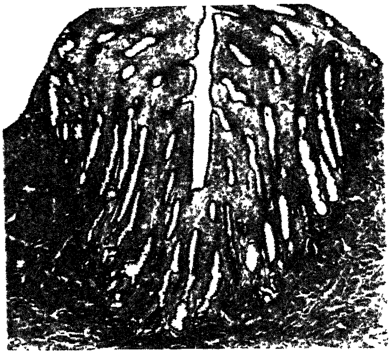

Fig. 5

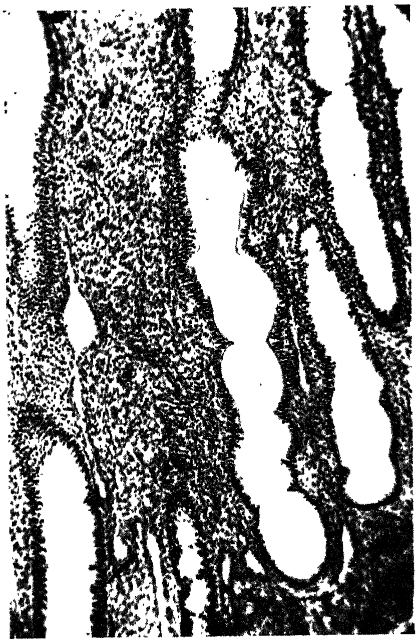

Fig. 6

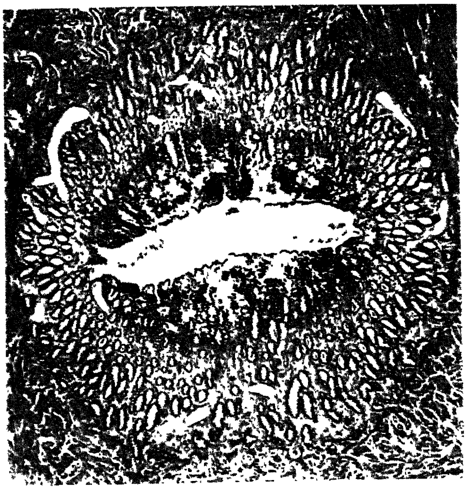

Fig. 9

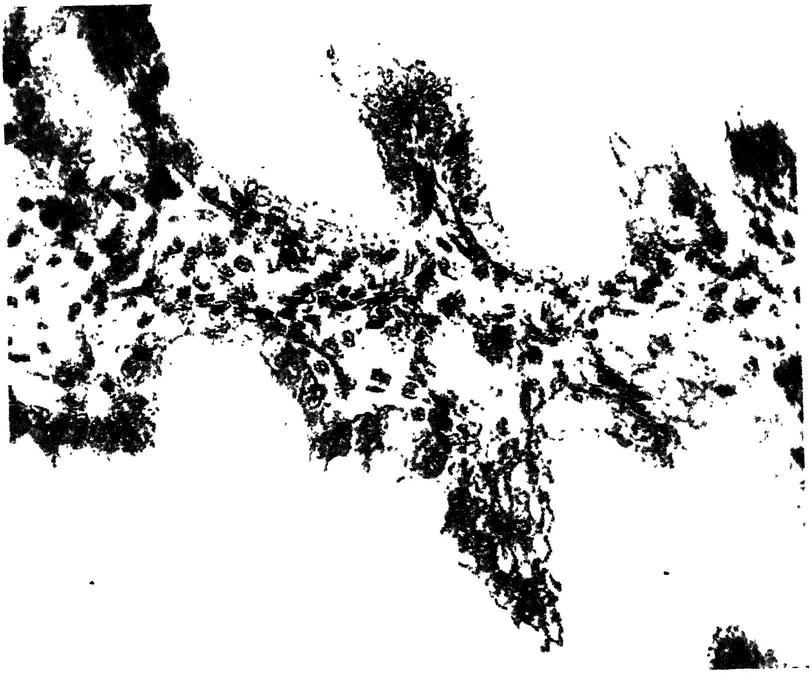

Fig. 8

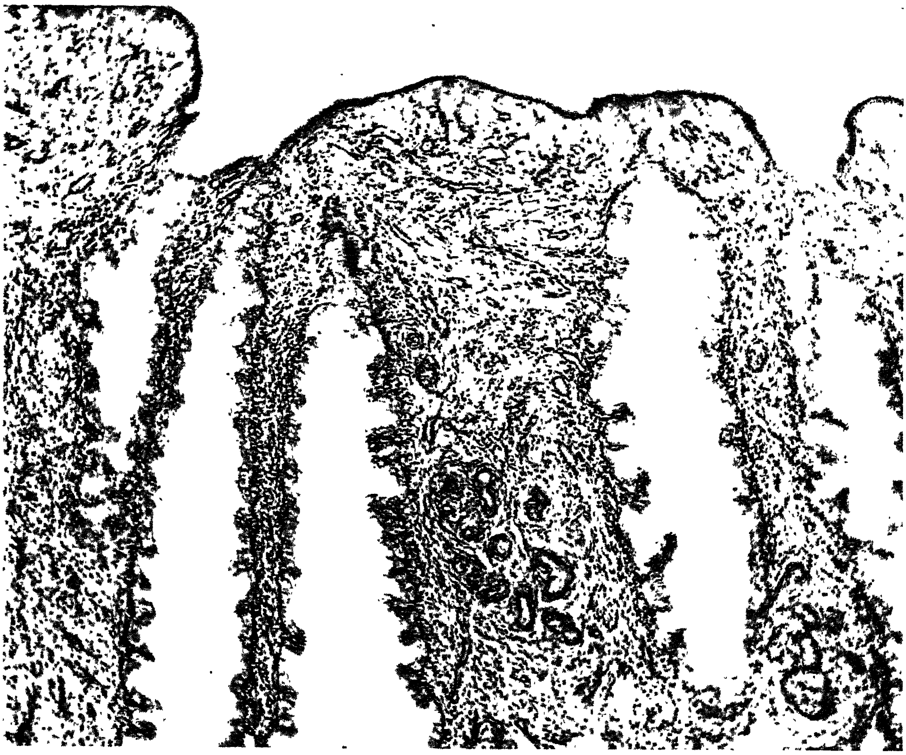

Fig. 7

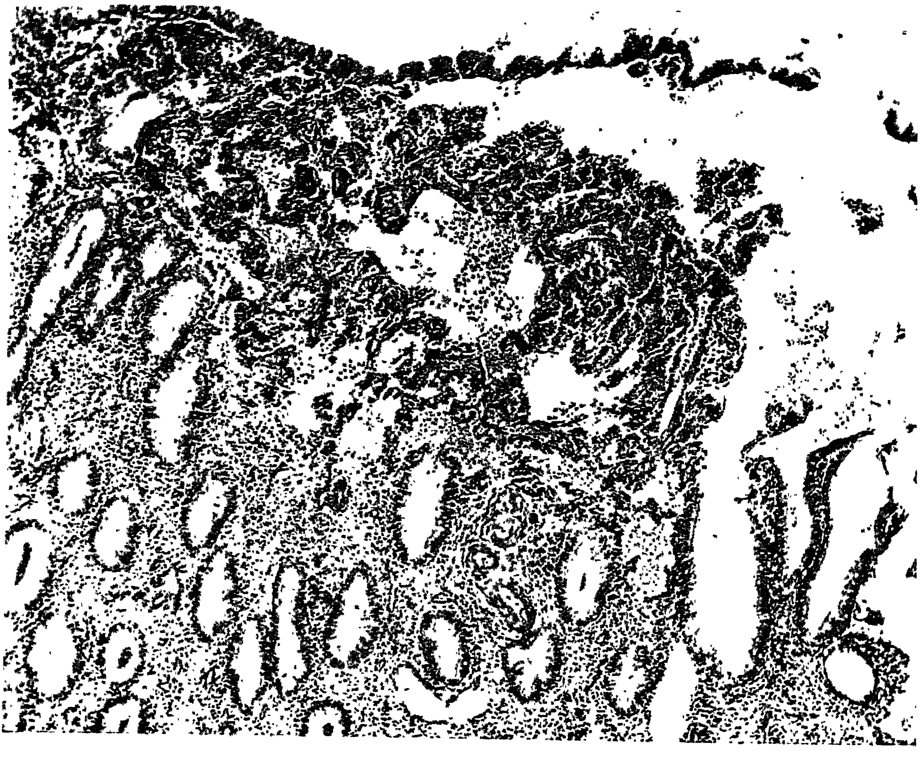

Fig. 10

TABLE I—SIMULTANEOUS ADMINISTRATION OF OESTRONE AND PROGESTERONE TO OVARIECTOMIZED RHESUS MONKEYS

		Phase 1			Phase 2				Phase 3			
			Oestrone Max. daily: total									
Exp. no.	Ref. no.	Days	I.U.	mg.	Days	Oestrone daily I.U.	Progesterone daily mg.	Nature of progestin	Days	Oestrone daily I.U.	Result	Control experiment
1	5·13	1–14	2000	1·90	15–26	50	2·00	O (2 R.U.)	27–29	500	Bleeding, 29th day	Bleeding, 25th day
2	3·1	1–14	3000	2·07	15–24	150	0·50	L (0·5 R.U.)	25–27	150	„ 27th day	Bleeding, 28th day
3	97·14	1–14	1000	1·04	15–26	100	1·00	O (1 R.U.)	27–28	100	„ 28th day	No bleeding, 40+6
4	64·11	1–14	3000	2·70	15–26	300	5·00	Cryst.	27–30	300	„ 30th day	„ 40+6
5	180·2	1–14	3000	2·70	15–26	500	10·00	Cryst.	27–30	500	„ 30th day	„ 40+5
6	3·3	1–14	3000	1·85	15–24	500	0·50	L (0·5 R.U.)	25–40	500	No bleeding, 40...	„ 40...
7	45·3	1–14	3000	1·95	15–24	500	0·90	B (1·5 R.U.)	25–40	500	„ 40+6	„ 40...
8	180·3	1–14	3000	2·70	15–26	1000	20·00	Cryst.	27–34	1000	Bleeding, 34th day	„ 40...
9	106·5	1–14	2000	1·82	15–40	50	1·50	O (1·5 R.U.)	—	—	No bleeding, 40...	Bleeding, 26th day
10	3·7	1–14	3000	1·95	15–27	150	0·90	B (1·5 R.U.)	—	—	Bleeding, 27th day	„ 28th day

O = Organon progestin; L = Leo progestin; B = British Drug Houses progestin; Cryst. = crystalline progesterone.

occurred in these experiments after the progestin injections were stopped may have been "oestrin-withdrawal bleeding". The processes leading to oestrin-withdrawal bleeding cannot be checked by the further administration of oestrin if a subthreshold level of oestrogenic stimulation has existed for more than a few days (Zuckerman 1937*a*). The second possibility is that the endometrial effects of luteal stimulation dominated those of oestrogenic stimulation in the present experiments. In this connexion it must be remembered that once the luteal stimulus that produces progestational changes is withdrawn, oestrone apparently cannot inhibit the consequent occurrence of uterine bleeding.

Progestational endometrial changes do in fact occur in certain circumstances during courses of treatment in which both oestrin and progestin are given together. Thus figs. 5 and 6 (Plate 7) show such changes in the uterus of an ovariectomized monkey, OM. 26, that was given both hormones. During the first 14 days of the experiment the animal was given oestrone in increasing amounts up to 3000 I.U. daily. From the 15th to the 25th day, when it was autopsied, it was given 150 I.U. and 1 R.U. of progestin (= 1 mg. progesterone) daily. The photomicrographs reveal the characteristic signs of progestational endometrial change. The epithelium is tall, and the glands are becoming dilated, coiled and sacculated. A more advanced phase of progestational change is shown in figs. 7 and 8 (Plates 8 and 7), which illustrate the condition of the endometrium of a monkey (106·5) that was given both oestrone and progestin for a longer period (for details see below). In both these animals the effects of the luteal hormone dominated those of the oestrone that was simultaneously administered, and had uterine bleeding been allowed to occur in these two animals, as in Exps. 3, 4, 5 and 8 of Table I, it would necessarily have had to be ascribed to the second and not to the first of the two possible explanations considered above.

On the other hand, figs. 9 and 10 (Plates 7 and 8) suggest that the uterine bleeding which occurs after the simultaneous administration of oestrin and progestin may be "oestrin-withdrawal" bleeding. The photomicrographs illustrate the endometrium of the monkey used in Exp. 4, the animal having been autopsied on the first day of bleeding. Although they are somewhat dilated, the glands are in general typical of the "interval" condition, and the picture of uterine bleeding provided is that of bleeding from an "interval" endometrium. The amount of progesterone which this animal received was far in excess of that normally required to transform an endometrium from the interval to the progestational type, and the fact that it was unable to do so in this instance, suggests that the hormone was rendered ineffective by its interaction with the oestrone that was being injected at the same time.* Similarly the fact that "oestrin-withdrawal" bleeding occurred (if it may be called that in view of the histological evidence) in spite of the daily administration of an amount of oestrone that is normally sufficient to maintain the endometrium in a phase of growth, suggests that the effective value of the oestrone was also greatly reduced. The following conclusions are therefore suggested by the experiments already considered:

(*a*) Progestational changes will occur in the endometrium when oestrin and progestin are given together (after a preliminary period of treatment in which oestrin is given alone), if the ratio of progestin to oestrin is sufficiently high.

(*b*) If the ratio is too low, progestational changes will not occur even though the absolute amount of progestin administered would be sufficient to produce these changes in other circumstances. In such cases, the phase of "interval" endometrial growth will be maintained only if the relative degree of oestrogenic stimulation remains above the threshold value for such stimulation.

(*c*) If progestational changes are produced, uterine bleeding will occur after the cessation of progestin injections in spite of the continued daily administration of a suprathreshold dose of oestrin.

(*d*) Bleeding will also occur when progestational changes have not been produced if the effective value of the oestrin that is given daily has been reduced to a subthreshold level through interaction with the luteal hormone. If the effective value is not reduced in this way (Exps. 6 and 7), bleeding will not occur.

Further light is thrown on these interactions by Exps. 9, 10 and 11, in which the period over which progestin was administered extended from the 15th day to the end of the experiment. Exp. 9 shows that the bleeding which would otherwise occur when an animal is injected daily with a subthreshold dose of oestrone may be inhibited if progestin is continuously administered in sufficient amounts (fig. 11). Figs. 7 and 8 (Plates 8 and 7) illustrate the condition of the endometrium in the animal used in this experiment at the end of the 26-day period in which progestin was given in conjunction with a subthreshold dose of oestrone. Progestational changes are very advanced, and fig. 8 reveals very clearly the characteristic fraying of the "progestational epithelium" that results from the dissolving out of glycogen during the process of staining (Campbell, Lendrum and Sevringhaus 1936). Exp. 10, on the other hand, shows that an inadequate daily amount of progestin will not prevent the occurrence of uterine bleeding when an animal is being injected daily with a threshold dose of oestrone.

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* Hisaw, Fevold and Meyer (1930) also found that if oestrin is given in "large amounts" at the same time as progestin, the effects of the luteal hormone are inhibited. Later, however, Hisaw (1935) reported that progestin can act on the primate endometrium in the presence of "large amounts" of oestrin.

It may be assumed that the amount of oestrone necessary to inhibit the endometrial effects of progesterone will vary from animal to animal and from time to time as the oestrone (and possibly the progesterone) threshold varies. Any attempt to define a quantitative ratio between the two hormones is further complicated by the fact that the inhibiting action is reciprocal, and that progesterone also reduces the effectiveness of oestrone. In Exp. 4 a large amount of progesterone was rendered ineffective by 1/170th its weight of oestrone, whose potency in turn was diminished by its interaction with the progesterone. In Exp. 10, progesterone in the approximate ratio of 60 parts to 1 part of oestrone failed to inhibit uterine bleeding that resulted from a lack of oestrogenic stimulation. On the other hand, progesterone in the ratio of 100:1 proved dominant in Exp. 3, and a ratio of 200:1 was similarly effective in Exps. 5 and 8.

Leonard and others (1932) report that 10 R.U. of oestrin will inhibit the endometrial effects of 1 R.U. of progestin in rabbits, and Allen and Meyer (1935) write that the same amount of progestin inhibits the cornifying effects of 6 R.U. of oestrin on the vagina of rats. More recently Robson (1936) has found that the effects of progesterone on the rabbit uterus are completely inhibited by 1/75th of its weight of oestrone and that oestrone will also inhibit slightly in a ratio of 1 to 300. The observations on monkeys reported above are thus in keeping with these findings on non-primate mammals.

DISCUSSION

The gonadotropic hormones of the pituitary influence the endometrium indirectly by way of the ovaries, and so far as is known the endometrial cycle is under the direct control of the gonadal secretions. Consequently, if the basis of reproductive periodicity and of the cyclical changes in the ovary are excluded from the immediate problem, it becomes necessary to explain the endometrial changes of the menstrual cycle in terms of the interaction of ovarian hormones. It was pointed out in the preceding part of this series of papers (Zuckerman 1937*b*) that the wave of oestrogenic stimulation follows the same course in anovular menstrual cycles as in cycles in which ovulation occurs. Experimental data were presented there which showed that uterine bleeding can be made to occur in ovariectomized rhesus monkeys at approximately 4-weekly intervals if the animals are injected daily with oestrone in amounts that vary relatively according to the phases of the normal sexual-skin cycle in animals which display that character in a clear and stable way, i.e. if oestrone is administered in increasing amounts for 14 days, and if the amount is then reduced to a threshold level. Since uterine bleeding occurs in normal animals at regular menstrual intervals whether or not ovulation takes place, such a wave of oestrogenic stimulation must be regarded as the basic mechanism of the endometrial cycle. The experiments reported in the present paper show that a phase of luteal activity can be superimposed upon a threshold level of oestrogenic stimulation without affecting the periodicity of uterine bleeding. It is therefore suggested that a corresponding interaction of ovarian hormones underlies the changes that occur in the luteal phase of normal menstrual cycles.

In such cycles the corpus luteum degenerates, and progestational stimulation ceases, a few days before menstruation sets in. If, as fig. 11 shows (Exp. 9, Table I), the progestational stimulus is prolonged, uterine bleeding is inhibited. This finding parallels Corner's observation (1935; also Corner and Allen 1936) that the continuous daily administration of 1 mg. of progesterone to rhesus monkeys will inhibit normal menstrual bleeding. Other experiments reported above show that progestin and oestrin must be perfectly balanced quantitatively for normal progestational changes to take place and for uterine bleeding to occur at 4-weekly intervals.

The hormone relations represented in fig. 3, according to which oestrogenic stimulation is succeeded by a ten or twelve-day period in which luteal and threshold oestrogenic stimulation are combined, thus reveal a mechanism of ovarian-hormone interaction which satisfies the conditions that are to a large extent defined by the normal endometrial cycle. This experimental finding bears out the interpretation of the menstrual cycle that was suggested in the first part of this series of papers (Zuckerman 1930) on the basis of observational data: "the follicular phase of the primate cycle, though its main effects occur in the earlier half, is prolonged over the entire cycle, and pseudo-pregnant growth is *superimposed upon* a persisting pro-oestrous endometrium". Two modifications of this hypothesis which are suggested by recent experimental work do not affect the main issue. The first is that the threshold level of oestrogenic stimulation in the second half of the cycle may be effectively lowered as a result of the action of the luteal secretions. The second is that the threshold for oestrogenic stimulation of the uterus varies cyclically (Zuckerman 1937*b*, *c*; probably as a result of changes, controlled by the adrenal cortex, in its water metabolism, Long and Zuckerman 1937), and that the level of stimulation not improbably suffers a further fall at the end of each cycle because of a rise in the endometrial oestrin threshold. Figs. 12A, B, which are based on the findings reported both in the present and in the preceding part of this series of papers, allow for this possibility, and give a diagrammatic representation of the endocrine interactions that appear to underlie (*a*) the endometrial changes in anovular menstrual cycles and (*b*) the endometrial changes of cycles in which ovulation occurs.

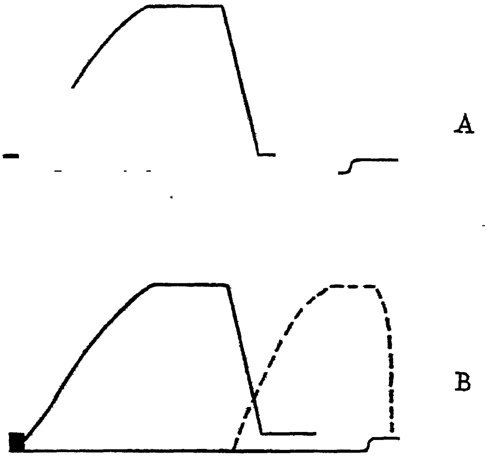

FIG. 12—Diagrammatic representation of the phases of the primate menstrual cycle. Unbroken line: the variation in the degree of oestrogenic stimulation. Broken line: variation in the degree of luteal stimulation. Base line: oestrin threshold. A, cycle in which ovulation does not occur; B, cycle in which ovulation occurs.

My best thanks are due to Dr. A. S. Parkes, F.R.S., both for his interest and for providing the first supplies of progestin used in this study. For further supplies of progestin I am greatly indebted to Dr. Macbeth and Dr. Tausk of Organon, Ltd., and to Dr. Schoeller of Schering, Ltd. I wish also to acknowledge gratefully the help of Mr. A. N. Guthkelch in the giving of injections. The monkeys were bought with the aid of a grant from the Medical Research Council.

SUMMARY

1. If oestrone is injected daily for 14 days into an ovariectomized rhesus monkey in increasing amounts (e.g. up to 3000 I.U. daily), and if the daily dose is then lowered to some point within the threshold range (e.g. 100 I.U.), uterine bleeding will occur about the 28th day of the experiment. The time of bleeding is not significantly affected if progestin is administered daily

during the first 10 or 12 days of the period in which the animal is being injected with a threshold dose of oestrone.

2. Even if the level to which the oestrone is lowered on the 15th day is above the threshold range, progestational changes will occur in the endometrium if the ratio of progestin to oestrin is sufficiently high. If the ratio is too low, progestational changes will not occur, even though the amount of progestin administered would be sufficient to produce these changes in other circumstances.

3. If progestational changes are produced, uterine bleeding will occur after the cessation of progestin injections even though the amount of oestrone administered daily is well above the threshold level.

4. Bleeding will also occur when progestational changes have not been produced if the effective value of the oestrone given daily has been reduced to a subthreshold level through interaction with the luteal hormone. If the effective value is not reduced in this way, bleeding will not occur.

5. These findings provide an experimental demonstration of an hypothesis of the menstrual cycle that was put forward in the first part of this series of papers on the basis of observational data.

REFERENCES

Allen, W. M. and Meyer, R. K. 1935 *Anat. Rec.* **61**, 427.

Campbell, R. F., Lendrum, F. C. and Sevringhaus, E. L. 1936 *Surg. Gynec. Obstet.* **63**, 724.

Corner, G. W. 1935 *Amer. J. Physiol.* **113**, 238.

Corner, G. W. and Allen, W. M. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **34**, 723.

Engle, E. T., Smith, P. E. and Shelesnyak, M. C. 1935 *Amer. J. Obstet. Gynec.* **29**, 787.

Hisaw, F. L. 1935 *Amer. J. Obstet. Gynec.* **29**, 638.

Hisaw, F. L., Fevold, H. L. and Meyer, R. K. 1930 *Anat. Rec.* **47**, 300.

Hisaw, F. L. and Leonard, S. L. 1930 *Amer. J. Physiol.* **92**, 574.

Leonard, S. L., Hisaw, F. L. and Fevold, H. L. 1932 *Amer. J. Physiol.* **100**, 111.

Long, C. N. H. and Zuckerman, S. 1937 *Nature, Lond.*, **139**, 1106.

Robson, J. M. 1936 *J. Physiol.* **88**, 100.

Smith, P. E. and Engle, E. T. 1932 *Proc. Soc. Exp. Biol., N.Y.*, **29**, 1225.

Zuckerman, S. 1930 *Proc. Zool. Soc. Lond.* p. 691.

— 1936 *J. Physiol.* **86**, 31*P*.

— 1937*a* *Proc. Roy. Soc.* B, **123**, 441.

— 1937*b* *Proc. Roy. Soc.* B, **123**, 457.

— 1937*c* *J. Physiol.* **89**, 49*P*.

DESCRIPTION OF PLATES 7 AND 8

FIG. 5—Endometrium of OM. 26. The animal was first injected for 14 days with increasing amounts of oestrone (up to 3000 I.U. daily). During each of the succeeding 10 days it was given 150 I.U. of oestrone and 1 R.U. of progestin. The mucosa is in an early progestational condition. The glands are dilated, sacculated and coiled. × 13.

FIG. 6—OM. 26. A higher-power view of some of the uterine glands. × 80.

FIG. 7—Endometrium of OM. 106. After 14 days' treatment with oestrone alone, this monkey was given 1½ R.U. of progestin and 50 I.U. of oestrone daily for 26 days. The mucosa is in an advanced progestational condition. × 82.

FIG. 8—OM. 106. High-power view, showing characteristic fraying of glandular epithelium of progestational endometrium due to dissolving-out of glycogen during the process of staining. × 370.

FIG. 9—Endometrium of OM. 64. Details about the treatment this animal received are given in Table I, Exp. 4, and on p. 157. Uterine bleeding is taking place from an interval endometrium in spite of the fact that the animal had been injected daily for 12 days with 5 mg. of progesterone. The effects of the progesterone were inhibited by the simultaneous daily administration of 300 I.U. of oestrone. × 11.

FIG. 10—OM. 64. High power view, showing the disorganization of the endometrium and the characteristic interval glands. × 89.

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Some Features of the Calcium Metabolism of the Shore Crab (*Carcinus maenas* Pennant)

By JAMES D. ROBERTSON
Carnegie Scholar

From the Sub-Department of Experimental Zoology, Cambridge, and the Marine Station, Millport

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I—INTRODUCTION

One of the features of the Arthropoda is the possession of a stiff outer covering of chitin secreted by the epidermis. This outer covering is cast periodically, and it is a characteristic of the group that increase in size takes place immediately after ecdysis or moulting of the old skin. During this period the new skin is soft and extensible.

In many of the higher Crustacea, this chitinous covering is hardened by the deposition in it of calcium salts, chiefly the carbonate and phosphate, thus forming a protective exoskeleton. Under this term are included the endophragmal skeleton and the partially calcified foregut.

The present work is a contribution to some aspects of the calcium metabolism of the shore crab, *Carcinus maenas*, which was used exclusively in the following researches. Investigation has been made of the moulting process, including the hardening of the soft new skin with calcium salts, and also of the permeability of the crab to the calcium ions of sea water. Apart from some isolated analyses of Irvine and Woodhead (1889), Schönborn (1912), and Hecht (1914), practically the only studies we have of calcium metabolism in the Brachyura are the papers of Paul and Sharpe (1916) and Drach (1935). Bethe (1928) has done some experiments on the permeability of *Carcinus* to the ions of sea water.

II—METHODS

For the permeability experiments, crabs were kept in different artificial sea waters. Artificial sea water of normal calcium content was made up from the data of Allen (1914) based on the analyses of Dittmar. By decreasing or increasing the amount of calcium chloride and adjusting the amount of sodium chloride correspondingly, sea waters were obtained with the same total concentration of salts but with different concentrations of calcium ions. Thus a 100% increase in the calcium concentration caused only a 4·5% decrease in the sodium concentration. By keeping crabs in such sea waters, osmotic pressure changes are avoided as pointed out by Bethe.

Blood was collected in a pipette through the arthrodial membranes at the bases of the legs. The cells were allowed to aggregate, and after centrifugation the clear plasma was used for analysis. Calcium was determined on 1 or 2 c.c. of plasma by the micro-permanganate titration as used by Van Slyke and Sendroy (1929), and protein gravimetrically (Peters and Van Slyke 1932, p. 688). The method of Kirk and Moberg (1933) was used for the determination of calcium in sea water, but the precipitations were made with brom cresol green instead of brom cresol purple as indicator. Some estimations were also done on 2 c.c. samples by the micro-method as for blood but with two precipitations. Magnesium was determined by the method of Washburn and Shear (1932), and chloride by silver nitrate titration with potassium chromate as indicator.

Greenberg and Gunther's (1930) technique was used for the ultrafiltration experiments, but the membranes were made of pyroxylin. The plasma in the sacs was connected to a carbonate buffer solution of *p*H 7·6 of approximately the same vapour pressure.

The secretion of the antennary gland was obtained by means of a glass cannula, and it was dry ashed to destroy interfering organic matter before estimation of its calcium content.

In order to make clear any changes in the relative composition of the tissues of crabs at different stages of the moult cycle, each specimen analysed was separated into three parts which were dried at 105° C., dry ashed, and analysed separately for calcium and phosphorus. The three parts were (1) the whole exoskeleton, (2) the hepatopancreas, and (3) all the other tissues including the blood, muscle, gonad, epidermis and dermis. The calcium was estimated volumetrically after precipitation as the oxalate with brom cresol green as indicator, and filtration in the filter tubes of Kirk and Schmidt (1929). Embden's (1921) gravimetric method was used for the phosphorus estimations, the factor being determined for different amounts of standard phosphate solutions. Carbonate was determined by gravimetric estimation of the carbon dioxide. Wet ashing of the integument with perchloric acid gave results identical with those obtained by the dry ashing method.

The chemical methods were tested with known standard solutions and pure calcium carbonate, the average deviation of results from the theoretical being 0·3 and 0·8 % for the macro- and micro-calcium methods respectively, and 0·5 % for both the phosphorus and the carbonate methods.

All the shore crabs used for experiments or analyses were fairly large male specimens (with a few stated exceptions), which had been collected or trawled not more than 48 hours previously. The crabs were not fed during any of the experiments.

III—THE PERMEABILITY OF *CARCINUS MAENAS* TO THE CALCIUM IONS OF SEA WATER

That the boundary membranes of *Carcinus* are permeable to the salts or constituent ions of sea water is at once suggested by the correspondence between the ionic composition of its blood and the surrounding sea water as shown by the analyses of Bethe.

The possible permeability of *Carcinus* to calcium ions was investigated, the data in Table I being obtained by keeping crabs in sea waters in which the calcium concentration varied between 11·55 and 130·50 mg./100 c.c. for a little more than a week. The crabs in each sea water were different specimens. Any permeability to calcium ions would cause a diffusion of ions across the boundary membranes, from a region of high concentration to one of lower concentration. Whether this had taken place or not was determined by chemical analysis of the blood and the external sea water after definite intervals. Equilibrium had apparently been established at the end of the duration of the experiments.

TABLE I—CALCIUM VALUES IN BLOOD AND IN SEA WATER

Blood	mg. Ca/100 c.c.						
Crab 1	18·99	26·27	47·80	61·08	62·19	109·56	124·73
2	22·42	25·09	47·59	59·87	62·38	94·28	114·38
3	16·62	33·78	48·06	55·83	65·30	89·34	112·74
4	—	—	54·85	54·28	59·88	91·03	116·20
5	—	—	—	49·15	60·09	93·29	—
Av.	19·34	28·38	49·58	56·04	61·97	95·50	117·01
Sea water	11·55	19·09	41·73	44·24	48·62	86·90	130·50

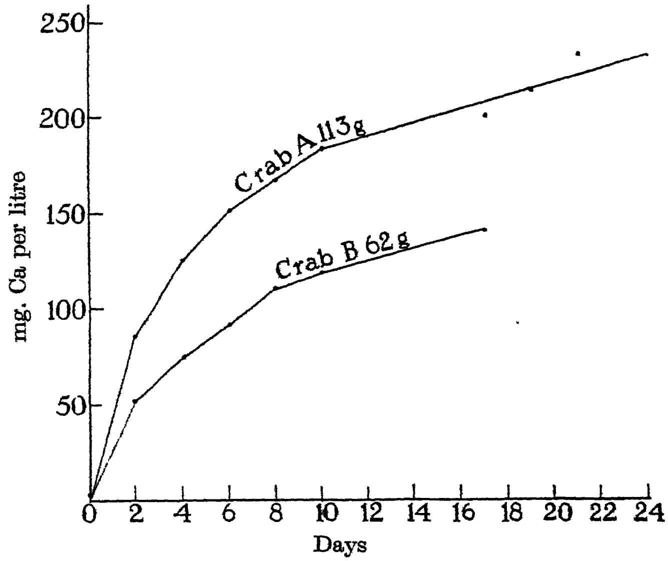

FIG. 1—Outward diffusion of calcium ions. Increase in calcium concentration of sea water, demonstrating loss from crabs in calcium-free sea water. (Corrected for evaporation and samples withdrawn.)

In sea waters of normal calcium concentration (42 mg./100 c.c.) and all the others except the one with the highest concentration, the blood calcium is kept at a higher level than the sea water calcium. The boundary membranes would appear to be permeable to calcium ions, since alteration of the external calcium concentration alters that of the blood correspondingly.

When crabs were kept in calcium-free sea water, large amounts of calcium diffused into it, far larger amounts than could possibly be present in the blood. This was first observed by Bethe. Thus the sea waters above, which had finally values of 11·55 and 19·09 mg./100 c.c., were initially calcium-free. Fig. 1 shows the amount of calcium which diffused into calcium-free sea water in the case of two crabs which were kept separately in 1 litre. The loss of calcium into the water, at first rapid, fell off, but equilibrium had not been attained even after 24 days in one case, during which 234 mg. had passed out from the crab. This large amount must have come from the tissues.

Under normal conditions there is probably an equilibrium between the sea water, the blood and the tissues. In the above experiment this equilibrium has been disturbed, and calcium diffuses out from the blood into the calcium-free sea water. To compensate for the lowered calcium concentration in the blood, calcium has been withdrawn from the tissues, and the two processes of outward diffusion and withdrawal of calcium will go on until a steady equilibrium is attained.

IV—THE DIFFUSIBLE CALCIUM

From dialysis and ultrafiltration experiments, it has been found possible to separate the calcium in the blood of vertebrates into two fractions, diffusible and non-diffusible. The non-diffusible calcium is supposed to be bound in some way to the colloids of the blood.

TABLE II—THE DIFFUSIBLE CALCIUM

		Calcium (mg./100 c.c.)			
Crab	Protein g./100 c.c. blood	Sea water	Blood	Ultrafiltrate	% diffusible
Hard	7·92	44·04	62·77	51·53	82
Hard	8·32	44·04	65·62	54·17	83
Hard	1·58	44·04	48·44	44·92	93
Hard	4·08	44·04	55·71	47·34	85
Hard	5·22	38·76	48·37	38·22	79
Hard	5·04	38·76	45·83	35·04	76
Hard	4·81	38·76	42·64	37·39	88
Hard	6·67	38·76	46·28	38·41	83
Hard	6·48	35·62	48·20	40·13	83
Hard	6·00	35·62	44·91	37·05	82
Hard	5·42	35·62	42·99	36·30	84
Soft	1·71	32·59	21·27	20·61	97
Hard	6·14	30·81	50·16	41·17	82
Soft	0·82	30·81	35·13	33·69	96
Soft	1·07	30·81	32·29	30·03	93
Soft	1·08	30·81	33·10	30·77	93

The diffusible calcium in the blood of specimens of *Carcinus maenas* was determined using Greenberg and Gunther's ultrafiltration technique, and determinations were also made of the protein content of the plasma and of the calcium content of the sea water in which the crabs were living (Table II). No correction has been made for the higher water content of the ultrafiltrate which is about 3 % higher than that of the blood plasma.

It will be noticed that in the hard crabs the diffusible calcium amounts to about 83 % of the total calcium. The protein content of the blood of the postmoult crabs is relatively low, 0·82–1·71 g./100 c.c., due to the dilution of the blood with water at the moult. The diffusible calcium is correspondingly increased to about 95 %. It is rather attractive to suggest that it is the diffusible calcium which is in equilibrium or approximate equilibrium with the sea-water calcium, and that the ordinary higher value of calcium in the blood plasma as compared with the sea water is due to the non-diffusible fraction bound to the colloids of the blood. There is yet, however, insufficient evidence to support this suggestion.

V—LEVEL OF CALCIUM IN BLOOD AND ANTENNARY GLAND SECRETION

It is possible that the antennary gland plays some part in controlling the ionic composition of the blood. In Table III the concentration of calcium in the antennary gland secretion has been compared with that of the blood plasma of the same specimens. In normal sea water its value is almost the same as the plasma value. In calcium-rich sea water the same is true, while in calcium-poor sea water it is slightly higher.

TABLE III—CALCIUM VALUES IN BLOOD AND ANTENNARY GLAND SECRETION

Sea water	Blood	Antennary gland secretion	Difference %
44·24	61·08	60·00	− 1·8
44·24	59·87	60·95	+ 1·8
44·24	54·28	61·85	+13·9
44·24	49·15	48·98	− 0·3
130·50	124·73	125·03	+ 0·2
130·50	114·38	114·71	+ 0·3
130·50	112·74	109·88	− 2·5
130·50	116·20	119·81	+ 3·1
11·55	18·99	21·23	+11·8
11·55	22·42	26·40	+17·8
11·55	16·62	18·92	+13·8

From Picken's (1936) work, there is evidence that the first stage in the formation of the excretory fluid may be a simple filtration of the blood. The diffusible calcium is, however, only about 83 % of the total calcium of the plasma. Since the concentration of salts in the plasma and antennary gland secretion are very similar (Picken), the high value of calcium in the secretion as compared with an ultrafiltrate cannot be due simply to a withdrawal of water. There would appear to be either an active secretion of calcium by the gland epithelium in addition to the filtration process, or the high value may be the result of some complicated ion exchange across the epithelium.

VI—CALCIUM BALANCE EXPERIMENTS

By keeping individual crabs in known volumes of sea water, one can ascertain whether they are excreting or absorbing calcium. In the following experiments each crab was kept in a glass vessel containing 1 litre of sea water which was aerated. At the end of the experiment (generally a week), the water was siphoned into a litre flask, and distilled water added to the mark to compensate for the evaporation which had taken place. Organic matter interfering with the calcium analysis was destroyed by ashing the first precipitate with nitric acid, or by removing it with trichloracetic acid before the analysis, a preferable procedure. Each experiment was repeated with the same crab for a second period, usually 6–8 days. The temperature of the water was 13–15° C. The experiments fall into three categories.

Experiments with ordinary hard crabs. It is seen from Table IV that crabs in the intermoult phase are excreting small quantities of calcium into the sea water. During the second week there is a reduction in the amount excreted. If it is assumed that the antennary gland is the only organ of excretion, and that no transference of ions has taken place across the boundary membranes, and further, that the calcium content of the antennary gland secretion is about 50 mg./100 c.c., one can calculate that the excretion has amounted to 0·6–2·8 mg. calcium per 50 g. weight per 24 hours, which is equivalent to a fluid secretion of 2·4–11·2 % of the body weight per day. The assumptions may not be wholly valid, but it seems reasonable to assume that most of the excretion has been through the antennary gland.

Experiments with soft postmoult crabs. In all the experiments with soft crabs, calcium ions were absorbed from the sea water in considerable amounts. When one specimen which had absorbed 63 mg. calcium from ordinary sea water was transferred to 50 % sea water for a week, it was able to absorb almost as much calcium (58 mg.) as it had done during its

TABLE IV—CALCIUM BALANCE EXPERIMENTS

Crab	Weight g.	Duration	Ca in sea water (mg.)		Excretion or absorption
			Initially	Finally	
Hard	89·61	7 days	414·3	421·9	Excr. 8
Hard	89·80	8	403·7	406·4	Excr. 3
Hard	60·74	7	414·3	427·3	Excr. 13
Hard	60·88	8	403·7	413·7	Excr. 10
Hard	67·70	7	414·3	441·4	Excr. 27
Hard	67·79	8	403·7	425·6	Excr. 22
Hard	71·17	7 in 100 % sea water	387·6	403·3	Excr. 16
Hard	71·95	7 in 50 % sea water	193·8	203·7	Excr. 10
Hard	71·44	7 in 50 % sea water	193·8	202·0	Excr. 8
Soft	56·28	7 in 100 % sea water	387·6	324·3	Abs. 63
Soft	55·73	7 in 50 % sea water	193·8	135·7	Abs. 58
Soft	55·67	7 in 50 % sea water	193·8	127·3	Abs. 67
Before and during moult (♀)	30·12	67 hr.	387·6	382·1	Abs. 6
After moult	38·40	7 days	387·6	316·3	Abs. 71
After moult	39·04	7	387·6	332·6	Abs. 55
Soft	37·72	7	387·6	349·6	Abs. 38
Soft	36·99	7	387·6	327·6	Abs. 60
Soft	52·41	7	387·6	300·6	Abs. 87
Soft	52·26	7	387·6	320·4	Abs. 67
Hard	112·59	8	148·9	210·5	Excr. 62
Hard	113·30	10	1·7	186·0	Excr. 184
Hard	113·04	14	186·0	235·8	Excr. 50
Hard	60·30	8	148·9	196·3	Excr. 47
Hard	61·70	10	1·7	120·0	Excr. 118
Hard	62·93	7	120·0	142·7	Excr. 23
Hard	94·90	8	797·3	779·4	Abs. 18
Hard	95·15	6	779·4	797·6	Excr. 18
Hard	71·00	8	797·3	790·6	Abs. 7
Hard	70·16	6	790·6	799·1	Excr. 9

stay in sea water of normal strength. The remarkable fact that soft crabs are able to absorb calcium ions from sea water, while hard crabs are passing calcium into the water, must be due to quite different conditions in the body fluids of the two stages.

Analysis of the blood calcium concentration of soft crabs, including some of the specimens which had actually absorbed calcium, showed the significant fact that the value in the blood was generally lower than that of the sea water in which they had been living (Table V). This is in direct contrast to the hard crabs, which (as shown in Table I) have blood calcium values higher than the surrounding sea water. In the soft crabs there are conditions for the inward diffusion of calcium from a region of high concentration, the sea water, to one of lower concentration, the blood. Since calcium is being continuously withdrawn from the blood to be deposited in the new integument, this diffusion process will not reach equilibrium, and further quantities of calcium will be absorbed. This ability to absorb calcium ions lasts for at least several weeks after ecdysis.

TABLE V—CALCIUM VALUES IN BLOOD OF SOFT CRABS AND IN SEA WATER

Crab	Calcium (mg./100 c.c.) Sea water	Calcium (mg./100 c.c.) Blood	Protein g./100 c.c. blood
Very soft *Carcinus*	38·76	32·20	—
Very soft *Carcinus*	38·76	33·17	—
Very soft *Carcinus* which had absorbed 60 mg. Ca the previous week	33·26	24·20	1·63
Very soft *Carcinus* which had absorbed 67 mg. Ca the previous week	32·59	21·27	1·71
Soft *Carcinus* in 50 % sea water which had absorbed 67 mg. Ca the previous week	12·95	19·19	1·05
Hard *Carcinus* in 50 % sea water which had excreted 8 mg. Ca the previous week	20·56	34·10	—

Experiments with calcium-rich and calcium-free sea waters. In calcium-rich sea water (about 80 mg./100 c.c.) during the first week small amounts of calcium were absorbed, but during the second week very similar quantities were excreted. Marked excretion of calcium into calcium-poor or calcium-free sea waters takes place. This has already been shown graphically (fig. 1).

VII—THE ABSORPTION OF WATER AT THE MOULT

The increase in size of the decapod Crustacea during or immediately after the moult is due to a rapid intake of water. It has been thought that the water is absorbed through the gut, but it is more likely that it is absorbed through the general surface of the crab. The work of Baumberger and Dill (1928) and Baumberger and Olmsted (1928) makes it probable that the absorption is an osmotic phenomenon.

TABLE VI—WATER CONTENT OF *CARCINUS*

Stage	Weight g.	% water	% water, cast skeleton included
Far from moult	92·0	67·9	—
Premoult (♀)	49·7	70·5	—
Average	—	69·2	—
Within 12 hr. after moult (♀)	20·0	87·6	82·7
Week after moult	87·9	86·7	81·1
Over week after moult	47·6	87·4	83·3
Fortnight after moult	64·9	85·6	80·4
Fortnight after moult	39·0	88·0	81·9
Carapace not fully hard	82·3	86·4	—
Average	—	87·0	81·9

In Table VI are given the water contents of specimens of *Carcinus maenas*. If the cast integuments are included in the body weight of the soft crabs, the water contents are lowered somewhat. From these data one can calculate the average amount of water absorbed during the moult:

%	Before moult	After moult
Water	69·2	81·9
Dry weight	30·8	18·1

Amount of water in crab of dry weight 30·8 g. after moult

$$= \frac{81{\cdot}9 \times 30{\cdot}8}{18{\cdot}1} \text{ g.}$$

$$= 139{\cdot}4 \text{ g.}$$

Therefore amount absorbed $= (139{\cdot}4 - 69{\cdot}2)$ g.

$$= 70{\cdot}2 \text{ g.}$$

Thus a crab of fresh weight 50 g. absorbs about 35 g. water.

The actual amount of water absorbed by two individuals was obtained by direct weighing before and after the moult (Table VII). There is good agreement between the amount absorbed and the calculated values. By analysing the sea water before and after the moult in the case of the second

TABLE VII—THE ABSORPTION OF WATER

Weight of crab g.	Weight of water absorbed at moult g. By weighing	Weight of water absorbed at moult g. By calculation
30·12	22·57	21·14
68·58	49·46	48·14

crab, it was found that both chloride and calcium ions had been absorbed, but in slightly different proportions from those existing in the sea water (Table VIII).

TABLE VIII—ABSORPTION AND EXCRETION OF IONS AT AND NEAR THE MOULT

Weight of crab g.	Period	Excretion (mg.) Ca	Excretion (mg.) Cl	Absorption (mg.) Ca	Absorption (mg.) Cl
66·11–68·58	1 week before moult	40	—	—	65
86·53	15 hr. during and after moult	—	—	26*	986*
88·49	2 days after moult	—	59	61	—

* In 49·46 g. sea water there are 21 mg. Ca and 910 mg. Cl.

VIII—RESORPTION IN THE INTEGUMENT BEFORE ECDYSIS

That there takes place some resorption of the mineral salts from the old integument before it is cast has long been known. It is evident that it must take place especially along the lines where the carapace splits apart from the rest of the skeleton. But Baumberger and Olmsted (1928) and Drach (1935) have found that much of the organic matter is also withdrawn.

An approximate measure of the amount of resorption can be obtained by comparing the weight and composition of a complete cast skeleton with the complete skeleton of an intermoult crab of identical size.

The data in Table IX show that there is a loss in weight of 39 % due to resorption of organic matter and salts. From the analyses it can be calculated that 79 % of the organic matter and about 18 % of the mineral salts have been withdrawn from the cast exoskeleton. Of the salts 24 % of the calcium, 21 % of the magnesium, 20 % of the carbonate, 37 % of the inorganic phosphate, and 74 % of the organic phosphorus have been resorbed. The latter has obviously been withdrawn with the organic matter.

TABLE IX—RESORPTION IN THE INTEGUMENT

Complete exoskeletons, carapacé width 3·8 cm.

	Hard skeleton	Cast skeleton
Dry weight	3·0954 g.	1·9006 g.
% loss in weight	—	38·6
Inorganic P	0·86 mg./100 mg.	0·88 mg./100 mg.
Total P	1·13	1·00
Ca	23·49	29·19
Mg	1·08	1·38
CO_2	25·19	32·65
Approximately:		
$CaCO_3$	52·87	68·58
$Ca_3(PO_4)_2$	4·30	4·40
$MgCO_3$	3·74	4·78
Organic P	0·27	0·12
Organic Ca	0·41	0·14
Total	61·59	78·02
Total salts	66·23	88·61

1000 mg. hard skeleton < 338 mg. organic matter / 662 mg. mineral salts

gives

614 mg. cast skeleton < 70 mg. organic matter / 544 mg. mineral salts

Most of the phosphorus and some of the calcium are probably stored in the soft tissues of the crab to be used afterwards for the substance of the new skeleton. It would also seem that considerable quantities of the resorbed calcium are excreted, since it was found (Table VIII) that a crab during the week previous to ecdysis had excreted 40 mg. calcium, an amount much larger than has been found to be excreted by intermoult hard crabs.

Relatively much more phosphorus than calcium is withdrawn, and consequently the Ca : P ratio of 14–21[*] : 1 in the normal skeleton increases to 22–33 : 1 in the cast skeleton (Table X). In general, the smaller the crab the less phosphorus it has in its integument. Table X also brings out the fact that before inorganic salts are deposited in the new skin there is present about 1 % of calcium and phosphorus probably mainly in organic combination. Within 12 hours of moulting carbonate has been deposited in the new skin and the percentage of calcium doubled. From this point, the phosphorus content increases and then falls off again, while the amount of calcium increases and the integument gradually hardens.

[*] The figure 21 is obtained from Table IX.

TABLE X—ANALYSES OF THE EXOSKELETON

Stage	Mg./100 mg. Ca	Mg./100 mg. P	Ca/P
Far from moult	24·5	1·46	17
Far from moult	(21·7	1·39	16)
Early premoult, new skin	1·1	1·02	1
Within 12 hr. after moult	2·4	1·09	2
Within 24 hr. after moult	(4·6	1·26	4)
Moulted week	8·0	1·74	5
Moulted over week	9·2	1·23	7
Moulted fortnight	7·9	2·40	3
Moulted fortnight	13·4	3·35	4
Moulted several weeks	16·6	0·95	17
Early premoult, old skin	25·5	1·76	14
Cast skeleton	28·0	1·29	22
Cast skeleton	28·2	1·05	27
Cast skeleton	30·8	1·38	22
Cast skeleton	30·5	0·92	33
Cast skeleton	28·3	1·17	24
Cast skeleton	(27·4	1·22	22)

( ) signifies analysis of skeleton minus legs.

IX—THE STORAGE OF CALCIUM AND PHOSPHORUS IN THE SOFT TISSUES

Paul and Sharpe (1916) have shown that there is a marked storage of calcium phosphate in the hepatopancreas of *Cancer pagurus* before the moult. After ecdysis the store is apparently used to harden the new exoskeleton.

In Table XI are given calcium and phosphorus analyses of the whole hepatopancreas and other soft tissues including the blood of a series of specimens of *Carcinus maenas* at different stages of the moult cycle. There is even more marked storage of these elements in *Carcinus* than in *Cancer*.

TABLE XI—THE STORAGE OF CALCIUM AND PHOSPHORUS

Stage	Hepatopancreas mg./100 mg. Ca	Hepatopancreas mg./100 mg. P	Hepatopancreas mg./100 mg. Ca/P	Other soft tissues mg./100 mg. Ca	Other soft tissues mg./100 mg. P	Other soft tissues mg./100 mg. Ca/P
Far from moult	9·82	7·34	1·3	1·17	1·12	1·0
Far from moult	9·08	6·43	1·4	—	—	—
Early premoult	12·10	8·27	1·5	1·24	1·09	1·1
Within 12 hr. after moult	2·92	3·68	0·8	0·55	0·88	0·6
Within 24 hr. after moult	4·85	5·87	0·8	—	—	—
Moulted week	0·98	5·47	0·2	0·45	0·97	0·5
Moulted over week	4·55	8·80	0·5	0·62	1·06	0·6
Moulted fortnight	5·94	4·89	1·2	0·59	0·95	0·6
Moulted fortnight	4·85	6·55	0·7	0·97	0·94	1·0
Moulted several weeks	0·36	3·67	0·1	1·08	0·79	1·4

In the hepatopancreas the percentage of calcium reaches 9–12 % and falls after the moult to 0·4 %. The phosphorus values change correspondingly. About 70 % of the total phosphorus in the hepatopancreas of an ordinary hard crab is in inorganic combination, and it is this inorganic phosphorus which decreases after the moult. Histologically, it is present as spherules, probably chiefly of calcium phosphate, which stain black with silver nitrate. The calcium content of the other soft tissues also falls immediately after the moult, but rises again to its normal value of about 1 % of the dry weight in a few weeks.

There can be no doubt that the stores of calcium salts in the tissues are used to harden the soft new integument, since calcification proceeds simultaneously with the depletion of the calcium reserves.

X—THE HARDENING OF THE INTEGUMENT

If the amount of calcium withdrawn from sea water by a newly moulted crab is determined for a certain period, and at the end of that period the tissues of the crab are analysed, one can calculate approximately the relative amounts of calcium which have come from the sea water and from the stored salts in the tissues.

Crab A: 88 g., analysed 1 week after moulting
Crab B: 65 g., analysed 2 weeks after moulting

	Calcium in mg.	
Source	Crab A	Crab B
Available from hepatopancreas	139	70
Available from the other soft tissues	47	28
Approximate amount in integument prior to calcification	15	12
Absorbed from sea water	187	172
Total	388	282
Actual amount in integument	354	309

The "available" calcium is the amount contributed by a tissue to the new integument, and is calculated for the hepatopancreas of crab A as follows:

% calcium in gland of premoult crab 12·10

Amount of calcium in gland of crab 1 week after moult = 12·25 mg.
= 0·98 %

Approximate amount of calcium in gland before moult $= \dfrac{12{\cdot}25 \times 12{\cdot}10}{0{\cdot}98}$ mg.
= 151·25 mg.

Available calcium = (151·25 − 12·25) mg.
= 139·0 mg.

The figure for the soft tissues is calculated in the same manner, knowing that the amount of calcium present after the week was 27·05 mg. = 0·45 %, and that the % of calcium in the tissues of a premoult crab is 1·24.

The agreement between the calculated amount of calcium present in the integument and the actual amount found by analysis is fair, considering the possible sources of error involved in the calculation. In the case of crab A, the amount available from the hepatopancreas may have been overestimated. One would expect both the calculated results to be low, since immediately after the moult the amount of organic matter particularly glycogen in the hepatopancreas and muscles decreases (Smith, 1913; Hoet and Kerridge, 1926), raising the calcium as percentage of the dry weight and lowering the calculated available amount.

The data show that over 50 % of the calcium in the new integument was absorbed from the sea water, the remainder coming from the stores in the tissues. The hepatopancreas supplied about twice as much calcium as the other soft tissues.

In accordance with present theory (Robison and Rosenheim, 1934), the enzyme phosphatase is believed to play a significant part in the calcification of bone. Norris and Rao (1935) and Engel and Chao (1935) have found this enzyme in the tissues and nerves of some invertebrates including the arachnid *Limulus*. The present writer has as yet been unable to find any phosphatase activity in extracts of the integuments of hard crabs or those in the process of calcification. While in bone there is about twice as much calcium as phosphorus, in the *Carcinus* exoskeleton there is 14–21 times as much calcium as phosphorus. This fact makes it improbable that any phosphatase mechanism is concerned in calcification in the shore crab.

The carbonate radical probably comes partly from the metabolic carbon dioxide of the crabs. While an absorption of the carbonate ion from sea water is not unlikely, the author has made no relevant experiments or analyses.

XI—Analyses of the Constituent Tissues

In Table XII are collected the absolute amounts of calcium and phosphorus in the different tissues of specimens of *Carcinus* at different stages of the moult cycle. The data as percentages of the dry weight have already been given in Tables X and XI. The resorption of phosphorus from the integument before it is cast is clearly shown by the analyses. Whereas in both the hard and early premoult crabs the phosphorus in the integument comprises about 59 % of the total amount in the crab, in the soft postmoult crabs the phosphorus in the cast integument only amounts to 26–38 % of the total amount in the crab inclusive of the cast skeleton.

TABLE XII—ANALYSES OF THE CONSTITUENT TISSUES

	Integument	Hepatopancreas	Other tissues
Dry weight (g.)	21·1322	1·5468	6·8814
Calcium (mg.)	5182·49	151·95	80·67
Phosphorus (mg.)	309·32	113·61	77·03

Far from moult: 92·03 g.; % Ca = 18·3; % P = 1·69

	Integument	New skin	Hepatopancreas	Other tissues
Dry weight	9·4114	0·9284	1·0420	3·2442
Calcium	2401·81	9·84	126·04	40·28
Phosphorus	165·30	9·47	86·15	35·20

Premoult (♀): 49·67 g.; % Ca = 17·6; % P = 1·96

	New skin	Hepatopancreas	Other tissues	Cast skeleton
Dry weight	0·5694	0·3690	1·5486	2·2128
Calcium	13·87	10·77	8·48	674·00
Phosphorus	6·20	13·59	13·68	20·43

12 hours after moult (♀): 20·02 g.; % Ca = 1·33; % P = 1·35;
including cast exoskeleton: % Ca = 15·05; % P = 1·15

	New skin	Hepatopancreas	Other tissues	Cast skeleton
Dry weight	4·4411	1·2496	5·9910	10·8636
Calcium	354·03	12·25	27·05	3071·00
Phosphorus	77·25	68·35	58·22	127·49

1 week after moult: 87·93 g.; % Ca = 3·37; % P = 1·74;
absorbed 187 mg. Ca from sea water

	New skin	Hepatopancreas	Other tissues	Cast skeleton
Dry weight	1·9302	0·9448	3·1260	3·7418
Calcium	178·38	42·99	19·53	1047·85
Phosphorus	23·67	83·11	33·02	48·14

Over week after moult: 47·55 g.; % Ca = 4·01; % P = 2·33

	New skin	Hepatopancreas	Other tissues	Cast skeleton
Dry weight	3·8866	1·1356	4·3462	8·0418
Calcium	308·94	67·45	25·77	2269·78
Phosphorus	93·13	55·48	41·10	84·47

Fortnight after moult: 64·91 g.; % Ca = 4·29; % P = 2·02;
absorbed 172 mg. Ca from sea water

	New skin	Hepatopancreas	Other tissues	Cast skeleton
Dry weight	2·1474	0·3458	2·1940	4·9006
Calcium	287·53	16·76	21·20	1511·10
Phosphorus	72·03	22·66	20·71	67·39

Fortnight after moult: 39·04 g.; % Ca = 6·94; % P = 2·46

	Integument	Hepatopancreas	Other tissues
Dry weight	4·6298	1·5424	4·9838
Calcium	766·94	5·57	53·81
Phosphorus	44·14	56·54	39·22

Moulted several weeks: 82·28 g.; % Ca = 7·41; % P = 1·25

The loss of the old skeleton causes the calcium percentage to fall from about 18 % of the dry weight to about 1–3 %, and the consistent rise in the percentage to 4 and 7 % after a fortnight is due mainly to the absorption of calcium and perhaps carbonate from the sea water. During this period no particulate food was available to the crabs, and consequently the loss in organic matter due to ordinary metabolism would tend to increase the percentage slightly.

Although under aquarium conditions lobsters eat their exuviae (Elmhirst 1930), the writer has never found the shore crab to do so. Indeed, complete cast skeletons are quite often to be found intact on the shore in the Clyde Sea-area during July and August. Undoubtedly crabs must obtain some calcium from their food, and the rapid hardening of the tips of the legs and the appendages will enable a soft crab to use food soon after the moult.

XII—Discussion

Before the work of Bethe, there was general agreement that the boundary membranes of marine invertebrates were semi-permeable, allowing the passage of water but not of the salts dissolved in the water. The work of Bethe (1928), Dakin and Edmonds (1931) and Koizumi (1932) affords strong evidence that marine invertebrates are permeable to the salts or constituent ions of sea water. Bethe found in *Carcinus* that when the concentration of calcium, magnesium, potassium and chloride was altered in the external sea water, a corresponding change occurred in the body fluids of the crabs. The interesting fact has been discovered by the writer that a new equilibrium between the external and internal concentrations of calcium is soon established, although the blood calcium is stabilized at a higher value than the sea-water calcium. This is clearly analogous to the conditions obtaining in dialysis experiments, where at equilibrium more calcium is present in a fluid containing colloids on one side of a collodion membrane than in the colloid-free dialysate on the other side.

Analyses showing the ionic composition of the antennary gland fluid and the blood of *Eriocheir sinensis*, an estuarine crab, have been given by Scholles (1933). The calcium in the gland secretion of individuals kept in sea water was on the average 6·5 % lower than the blood value. Bialaszewicz (1932) has shown that calcium, magnesium and sulphate are being actively excreted by the antennary gland of *Maia squinado* in proportions relatively greater than other ions such as chloride and potassium. This was found by comparing the secretion with an actual ultrafiltrate of the blood. By removing the antennary gland secretion as it was formed, he found that the amount excreted during 24 hours was between 2 and 3 % of the body weight. Nagel (1934) has estimated the amount of secretion formed in *Carcinus* by plugging the opening of the gland to the exterior and measuring the increase in weight. During 5 or 10 hour periods, the increase in weight was calculated to cause an increase in 24 hours of 10–17 % of the body weight. This increase would seem to be due to an absorption of water, and Nagel assumes that it must be balanced by the excretion of an equivalent amount of fluid by the antennary gland. There is some doubt about the validity of this assumption, and the discrepancies between the results of the two authors are very considerable. The present author's estimate of the amount of secretion is from 2 to 11 % of the body weight per day, and lies between the two other estimations.

The conspicuous feature of the moulting of crabs is their increase in size and weight as they emerge from the old integument. A specimen of *Carcinus* weighing 50 g. absorbs about 35 g. water at the moult. This can be compared with a corresponding value of 58* g. in *Maia squinado* (Drach 1936), and values of 17 and 22 g. in *Pachygraptus crassipes* and *Hemigraptus oregonensis* respectively (Olmsted and Baumberger 1923). The work of Baumberger and Olmsted, and Baumberger and Dill offers us a clue to this remarkable intake of water. It appears that just before the moult the osmotic pressure of the blood (as measured by its freezing-point depression) undergoes a considerable increase. Within 15 min. of emerging from the cast integument the osmotic pressure falls, indicating an osmotic intake of water. Whether the increased osmotic pressure is due to a concentration of the electrolytes in the blood or to an increase in the non-electrolytes or both is not known. The firmly established fact that the glycogen content of the crab as a whole and of both the hepatopancreas and muscles falls after the moult (see e.g. Schönborn 1912; Smith 1913; Hoet and Kerridge 1926) would suggest that its breakdown to sugar might be at least partly responsible for the increased osmotic pressure prior to the moult. Indeed Drilhon (1933) has found that in *Maia squinado* the amount of glucose in the blood immediately before moult may rise to 18 mg./100 c.c., while just after the moult it may fall to 6 mg.

While Baumberger and Olmsted have calculated from their osmotic pressure measurements that pure water is absorbed, the writer has found that at least calcium and chloride ions are absorbed with the water. Theoretically, if the increased osmotic pressure was due to non-electrolytes, one would expect at first an osmotic intake of pure water, and then diffusion inwards of ions, since the electrolytes in the blood would now be less concentrated than those of sea water. Actually, the two processes may be more or less simultaneous.

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* Calculated from Drach's figures.

Drach seems to have been the only person who has made a quantitative estimate of the amount of resorption. By comparing the dry weights of carapaces from cast skeletons with those from hard crabs judged to be near the moult, he has calculated that resorbed material in the carapace of *Carcinus* amounts to 22 % and in *Cancer* to about 20 % of the original dry weight. Further study of the carapace of *Cancer* showed that the resorption amounted to 56 % of the organic matter and nearly 10 % of the mineral salts. He also observed that certain zones in the endophragmal skeleton entirely disappeared before moult, thus making the process mechanically possible. I have confirmed this latter observation in *Carcinus* by comparing a cast skeleton with the skeleton of a hard crab obtained by boiling the specimen in sodium hydroxide to remove the soft tissues. I have also shown that there is relatively much more resorption of phosphorus than calcium. The phosphorus and perhaps some of the calcium is probably stored in the tissues, since higher values of these elements are found in a premoult crab than in an ordinary hard crab, and both are presumably deposited in the new integument after the moult.

It is probable that all the Brachyura store calcium salts in the soft tissues, particularly in the hepatopancreas, before moulting, and that these are used to harden the new integument. The first indication of this was found by Schönborn in *Maia squinado*, where the percentage of calcium in the hepatopancreas before the moult was about 3·8 % of the dry weight, and 1·5 % in a second specimen after the moult. Paul and Sharpe's work on *Cancer pagurus* was more convincing, and showed calcium values of 6 and 7 % falling to 0·01 after the hardening of the integument. Scholles gives a figure of 1·9 % in a very soft individual of *Eriocheir sinensis*. In *Carcinus* I have found the storage of calcium to be much greater, the percentage of calcium in the gland reaching 9–12, and falling eventually to 0·4, while the corresponding phosphorus figures were 6–8 and 3·7.

In the genera of Astacura and Anomura analysed so far, there is no storage at all (Paul and Sharpe 1916—*Homarus* and *Lithodes*; Yonge 1924—*Nephrops*). According to Elmhirst, marked deposits of calcium can be demonstrated histologically in the hypodermis of premoult lobsters, and these disappear after the moult. But the most peculiar calcium reserves in the Astacura are the gastroliths, plano-convex masses of calcium carbonate which appear in the foregut, one on each side several days before the moult. There can be no doubt that these represent material which has been resorbed from the exoskeleton. In *Astacus* (Damboviceanu 1932) they reach their maximum size, about 8 mm. in diameter, immediately before moulting. A few hours after the moult, the gastroliths decrease in size, and at the end of 2 days have disappeared. This progressive diminution in size and final disappearance is correlated with the hardening of the exoskeleton, and it is reasonably certain that the gastroliths are used as a source of calcium for the calcification of the new skin.

In the isopod *Ligia exotica*, Numanoi (1934) has shown that a large amount of calcium resorption takes place before the old skeleton is cast, but he found no storage of this element in the hepatopancreas.

Both Schönborn and Hecht gave analyses showing that the total amount of calcium in postmoult crabs was much less than that in hard individuals. Consequently, both these authors inferred that there must be absorption of calcium from the surrounding sea water in order that the hard state might again be attained. Irvine and Woodhead appear also to have believed this. While food is one obvious source of calcium, the writer has shown by analyses that the absorption of calcium from sea water does take place to a very considerable extent in soft specimens of *Carcinus*. Thus in two specimens analysed a week and a fortnight after the moult, more than 50 % of the calcium in the new integuments had come from the sea water.

I wish to thank Dr. James Gray for helpful advice during the course of this work. To the staff of the Marine Station, Millport, and particularly Mr. Elmhirst, I am indebted for the facilities which I enjoyed during my visits there. I am also indebted to the Managers of the Balfour Fund for a grant towards the collection and preparation of much of the material analysed here, and especially to the Carnegie Trustees for the Scholarship which made this work possible.

XIII—SUMMARY

1—*Carcinus maenas* is permeable to the calcium ions of sea water. A new equilibrium between the concentration of calcium in the body fluid and that in the sea water is established when crabs are placed in sea water of altered calcium content.

2—The presence of colloids in the blood accounts at least partly for the higher value of calcium in the blood as compared with the value in the sea water in which the crabs are living.

3—A specimen of *Carcinus* weighing 50 g. absorbs about 35 g. of water at the moult.

4—Resorption of much of the organic matter and some of the mineral salts takes place before the casting of the exoskeleton at the moult.

5—The stores of calcium and phosphorus in the soft tissues particularly in the hepatopancreas are used to harden the new skeleton.

6—Soft postmoult crabs absorb calcium ions from sea·water. This calcium is also used in the calcification of the new skeleton.

References

Allen, E. J. 1914 *J. Mar. Biol. Ass. U.K.* **10**, 417.
Baumberger, J. P. and Dill, D. B. 1928 *Physiol. Zoöl.* **1**, 545.
Baumberger, J. P. and Olmsted, J. M. D. 1928 *Physiol. Zoöl.* **1**, 531.
Bethe, A. 1928 *Pflüg. Arch. ges. Physiol.* **221**, 344.
Bialaszewicz, K. 1932 *Arch. int. Physiol.* **35**, 98.
Dakin, W. J. and Edmonds, E. 1931 *Aust. J. Exp. Biol. Med. Sci.* **8**, 169.
Damboviceanu, A. 1932 *Arch. Roum. Path. Exp. Microbiol.* **5**, 239.
Drach, P. 1935 *C.R. Acad. Sci., Paris*, **201**, 157, 1424.
— 1936 *C.R. Acad. Sci., Paris*, **202**, 1817.
Drilhon, A. 1933 *C.R. Acad. Sci., Paris*, **196**, 506.
Elmhirst, R. 1930 An Interim Report (Report on Crabs, 1930) of the Interdepartmental Committee on Crabs and Lobsters, Appendix 6. H.M. Stat. Office.
Embden, G. 1921 *Hoppe-Seyl. Z.* **113**, 138.
Engel, G. L. and Chao, I. 1935 *J. Biol. Chem.* **108**, 389.
Greenberg, D. M. and Gunther, L. 1930 *J. Biol. Chem.* **85**, 491.
Hecht, S. 1914 *Science*, **39**, 108.
Hoet, J. P. and Kerridge, P. M. T. 1926 *Proc. Roy. Soc.* B, **100**, 116.
Irvine, R. and Woodhead, G. S. 1889 *Proc. Roy. Soc. Edinb.* **16**, 324.
Kirk, P. L. and Moberg, E. G. 1933 *Industr. Engng. Chem.* **5**, 95.
Kirk, P. L. and Schmidt, C. L. A. 1929 *J. Biol. Chem.* **83**, 311.
Koizumi, T. 1932 *Sci. Rep. Tôhoku Univ.* Ser. IV, **7**, 259.
Nagel, H. 1934 *Z. vergl. Physiol.* **21**, 468.
Norris, E. R. and Rao, R. 1935 *J. Biol. Chem.* **108**, 783.
Numanoi, H. 1934 *J. Fac. Sci. Tokyo Univ.* Ser. IV, **3**, 351, 359.
Olmsted, J. M. D. and Baumberger, J. P. 1923 *J. Morph.* **38**, 279.
Paul, J. H. and Sharpe, J. S. 1916 *J. Physiol.* **50**, 183.
Peters, J. P. and Van Slyke, D. D. 1932 "Quantitative Clinical Chemistry", **2**, 688. Baltimore.
Picken, L. E. R. 1936 *J. Exp. Biol.* **13**, 309.
Robison, R. and Rosenheim, A. H. 1934 *Biochem. J.* **28**, 684.
Scholles, W. 1933 *Z. vergl. Physiol.* **19**, 522.
Schönborn, E. G. v. 1912 *Z. Biol.* **57**, 534.
Smith, G. 1913 *Quart. J. Micr. Sci.* **59**, 267.
Van Slyke, D. D. and Sendroy, J. 1929 *J. Biol. Chem.* **84**, 217.
Washburn, M. L. and Shear, M. J. 1932 *J. Biol. Chem.* **99**, 21.
Yonge, C. M. 1924 *Brit. J. Exp. Biol.* **1**, 343.

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The Effect of X-Rays on the First Meiotic Division in three species of Orthoptera

By M. J. D. White

University College, London

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[Plate 9]

1—Introduction

Although several papers have been published which deal with the cytological effects of irradiation, the whole subject is still in the preliminary or exploratory stage. It is clear that a complex sequence of biochemical phenomena result from the irradiation of a tissue, and that these may produce morphological changes in the chromosomes and other constituents of the cell. Generalizations based on the genetical results obtained by irradiating *Drosophila* sperm are dangerous, since the male gamete is a highly peculiar cell with practically no cytoplasm or nuclear sap. The few papers which have dealt with the effects of X-rays on the meiotic divisions (Helwig 1933; Mather 1934; Stone 1933; Huskins and Hunter 1935; Riley 1936), although interesting, are really only preliminary studies.

In a previous paper the author (White 1935) dealt with the effects of irradiation on the spermatogonial (mitotic) divisions of the migratory locust. This, the second paper, deals with certain effects observed at the first meiotic division in three other species of Orthoptera, *Mecostethus grossus* L., *Metrioptera brachyptera* L. and *Stauroderus* (*Chorthippus bicolor* Charp. Future papers will deal in further detail with other phenomena resulting from irradiation, the effects of different dosages, etc.

Of the three species dealt with here, *Mecostethus* and *Stauroderus* are members of the family Acrididae (short-horned grasshoppers) while *Metrioptera* is one of the Tettigonidae (bush-crickets or Katydids). Most of the work was done on *Mecostethus* and *Metrioptera*, but some fragmentary observations on *Stauroderus* are included in order to show that the phenomena described are essentially the same in at least three species of Orthoptera.

Mecostethus has eleven pairs of autosomes, *Metrioptera* fifteen, while *Stauroderus* has only eight. All the species are XO in the male. In *Mecostethus* and *Metrioptera* all the chromosomes, including the X, have nearly terminal spindle attachments, there being a minute "polar granule" beyond the spindle attachment. *Stauroderus* has five pairs of autosomes, together with the X, of this type, but the remaining three pairs of autosomes are V-shaped, with submedian spindle attachments.

All these species are excellent cytological material since the chromosomes are very large (especially in *Mecostethus*) and vary in size, so that individual autosomes can be identified in many cases.

2—Material and Methods

Male imagines of the three species were collected at Beaulieu Road, New Forest, and brought back to the laboratory for irradiation. *Mecostethus* and *Metrioptera* were irradiated simultaneously, in the same muslin bag, and consequently received the same dosage. The conditions of irradiation were as follows: Coolidge tube; distance 30 cm.; 65 kV; 5 mA; unscreened radiation.

The time of exposure was 5 min. except in the case of one batch of *Stauroderus* which received 20 min. exposure. An ionization-chamber was unfortunately not available so that no accurate measurements of the dosage in Roentgen units was possible; the 5 min. exposure, however, gave a calculated dosage of under 600 ν. The material was fixed in Flemming's fluid, sectioned at 25μ and stained by Newton's Gentian Violet method.

3—The First Meiotic Division in the Control Material

The normal course of meiosis in *Mecostethus grossus* has been described in detail by Janssens (1924) and by McClung (1927, 1928); I have recently added some data on chiasma localization and chiasma frequency (White 1936). The main features are (1) restriction of chiasmata in nearly all chromosomes to a short region near the spindle attachment, (2) the occurrence of a "diffuse stage" during which the chromosomes become very difficult to fix or stain and which replaces pachytene, (3) the absence of any secondary shifting ("terminalization") of chiasmata. The general features of the first meiotic division in unirradiated material are shown in fig. 1 *a–e*, and in fig. 2 *a* and *b*. There is one autosome (probably the VIIth in order of size, but it is difficult to be certain, as there are several which are almost the same length) which is strongly heteropycnotic in its distal

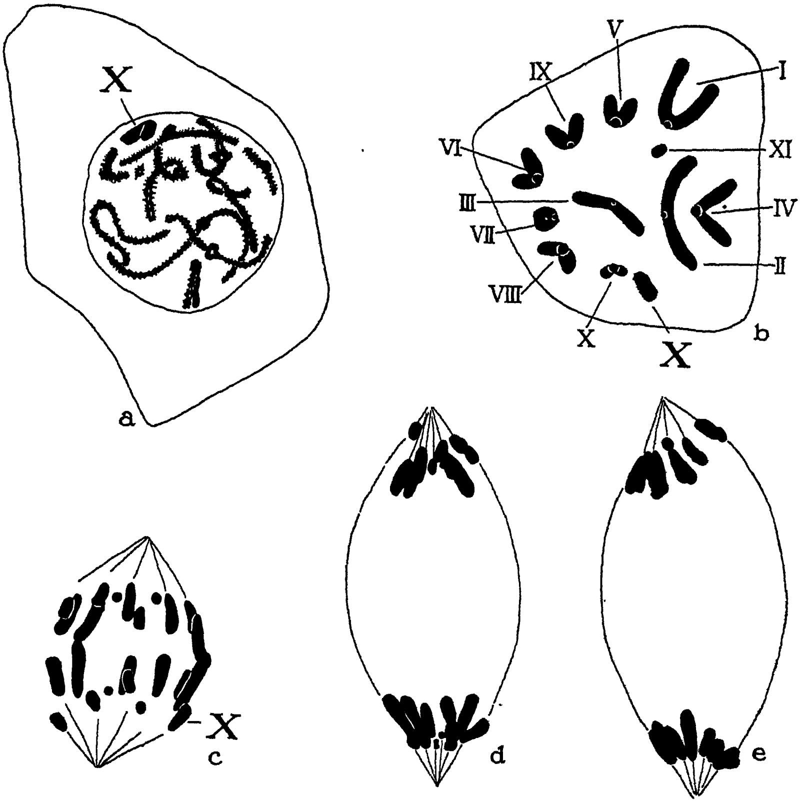

FIG. 1—The first meiotic division in *Mecostethus*; unirradiated material. a = diakinesis, b = first metaphase, c = mid anaphase, d and e = late anaphase. The bivalents in b are labelled I–XI in order of size. Only the VIIth shows two chiasmata. X = the X-chromosome.

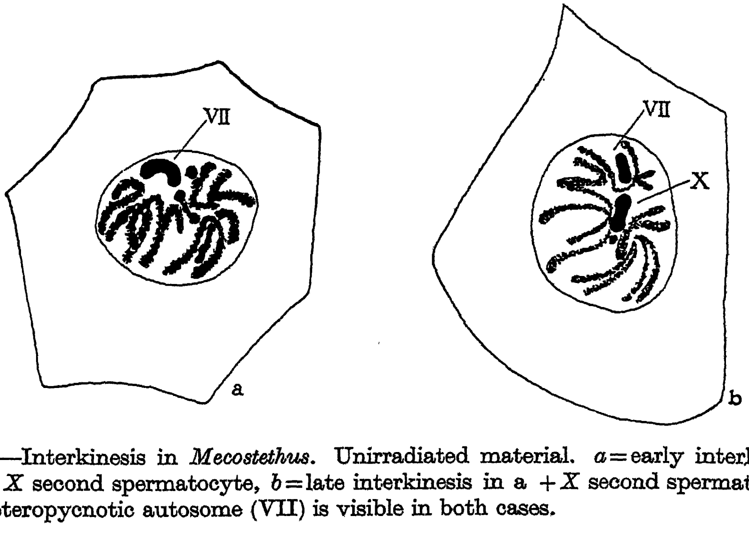

FIG. 2—Interkinesis in *Mecostethus*. Unirradiated material. a = early interkinesis in a $-X$ second spermatocyte, b = late interkinesis in a $+X$ second spermatocyte. The heteropycnotic autosome (VII) is visible in both cases.

half at diplotene-diakinesis and also at interkinesis (fig. 2). This autosome very frequently lies just next to the X during the prophase stages of the first division, so that one can presume that in all probability its heteropycnotic region is, at least in part, homologous to a region in the X; if this were so one would expect it to show a pairing-affinity with the X. It never actually forms a chiasma with the X, which is presumably due to the heteropycnosis which usually prevents chiasma-formation, even between two X's in a tetraploid spermatocyte (White 1933 and later unpublished observations). This heteropycnotic autosome (which I shall call the VIIth for the sake of convenience) is the only one which frequently forms a second chiasma; all the others, except in very rare cases, only form one, just next to the spindle attachment. In addition to the large distal heteropycnotic region in the VIIth autosome, there is a very short heteropycnotic region just next to the spindle attachment in all the autosomes; this region is about 1μ long at diplotene, i.e. somewhat shorter than the region to which the chiasmata are restricted, which extends a little beyond the heteropycnotic region. In view of what will be said later it is necessary to point out that anaphase-separation at the first meiotic division is extremely regular in *Mecostethus* and that the chromosomes composing the bivalents do not have any difficulty in getting free from one another. This is usual in organisms with a low number of chiasmata per bivalent.

The normal meiosis in *Metrioptera* is also characterized by chiasma-localization, at any rate in the larger bivalents (White 1936). There is no diffuse-stage and no special heteropycnotic regions in the autosomes. The X-chromosome is very large, and is usually attached to the spindle about midway between the equator and one of the poles; occasionally, however (fig. 9*d*), it may be attached in the equatorial plane. The separation of the chromosomes at the anaphase of the first meiotic division, although not quite so regular as in *Mecostethus*, takes place in normal material without any special difficulty, except that the larger chromosomes usually separate somewhat later than the small ones.

The normal meiosis of *Stauroderus bicolor* has been described by Darlington (1936) and I have not studied it in detail.

4—MEIOSIS IN X-RAYED *MECOSTETHUS GROSSUS*

The general appearance of the first meiotic division in individuals fixed 7¼ hr. after irradiation is shown in figs. 3 and 4 and in figs. 13 and 14, Plate 9. All stages up to prometaphase (fig. 3*a*) appear to be normal. The anaphases of the first meiotic division are, however, strikingly different

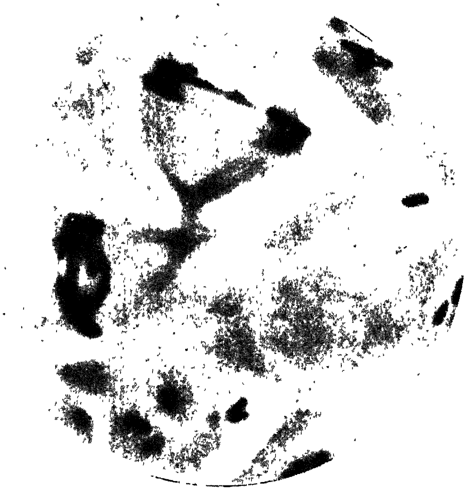

FIG. 12

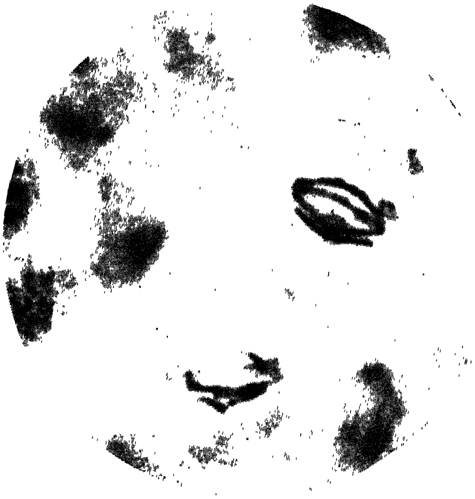

FIG. 13

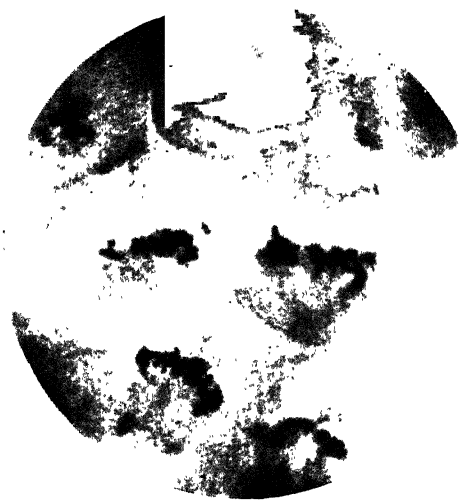

FIG. 14

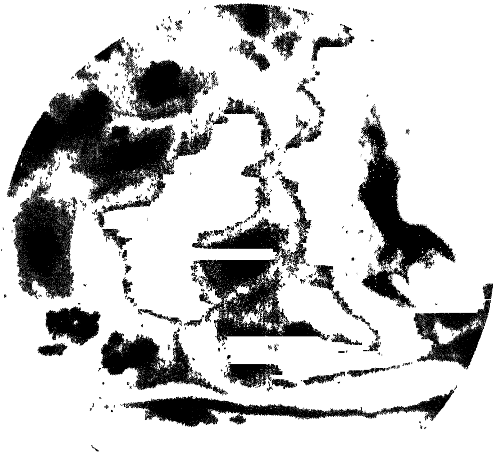

FIG. 15

from those seen in the control material. This difference may be described by saying that the chromosomes appear to have become viscous, so that they only separate with great difficulty, if at all. Thus in fig. 3*d* all the bivalents can be seen and there is no doubt that none of them has become fragmented; but the two chromosomes of which each bivalent is composed

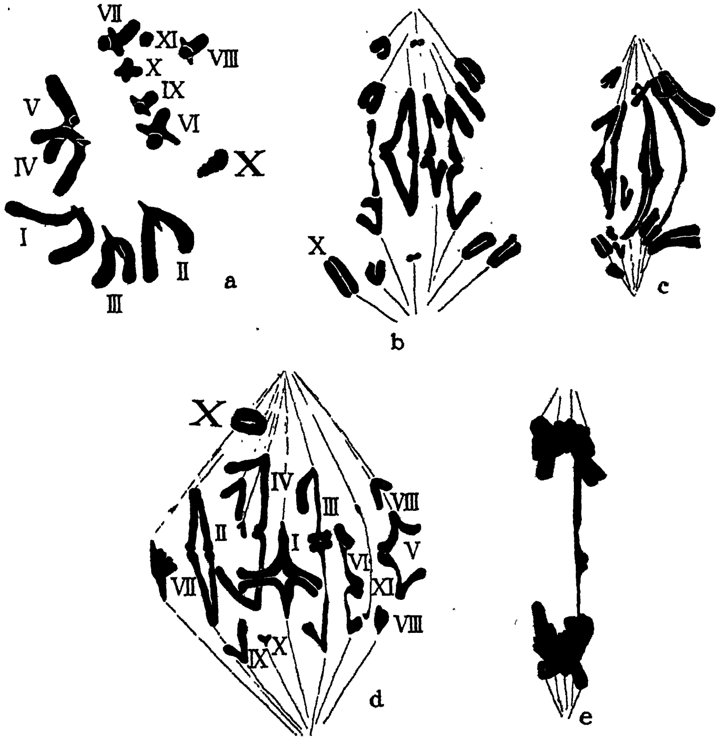

FIG. 3—The first meiotic division in irradiated *Mecostethus*. 7½ hr. material. *a* = prometaphase, all chromosomes normal, one chiasma in each bivalent; *b* and *c* = anaphases, the chromosomes separating with difficulty due to "stickiness" (not all bivalents drawn in these two figures); *d* = an early anaphase showing all the bivalents; *e* = late anaphase; the two groups of chromosomes are held together by a bridge of sticky chromatin.

(and which are held together by pairing on either side of a single chiasma) stick together in spite of the repulsion between the spindle attachments which is pulling them apart. The result is that frequently the two groups of anaphase chromosomes are held together by a "bridge" as in fig. 3*e*. It should be pointed out that these "bridges" are entirely different from those which have been described by Darlington (1936) and others as resulting from crossing-over in an inverted segment of a chromosome.

The anaphases illustrated in fig. 3, although abnormal for *Mecostethus*, are not unlike those seen in some organisms with many chiasmata per bivalent (e.g. *Triton*, Janssens 1909). Those illustrated in fig. 4 are, however, even more abnormal in that the different chromosomes have begun to fuse together laterally into a more or less amorphous mass. As a result of their "stickiness" they are prevented from separating completely from one another and usually give rise to a diploid restitution nucleus (fig. 5*a*). The degree to which the chromosomes are affected by this alteration in their physical consistency varies somewhat from cell to cell even in the same individual. It is probable that most of the anaphases in fig. 3 would give rise to two separate interkinesis nuclei (fig. 5*b*) while all those in fig. 4 would probably give rise to restitution nuclei of the type shown in fig. 5*a*.

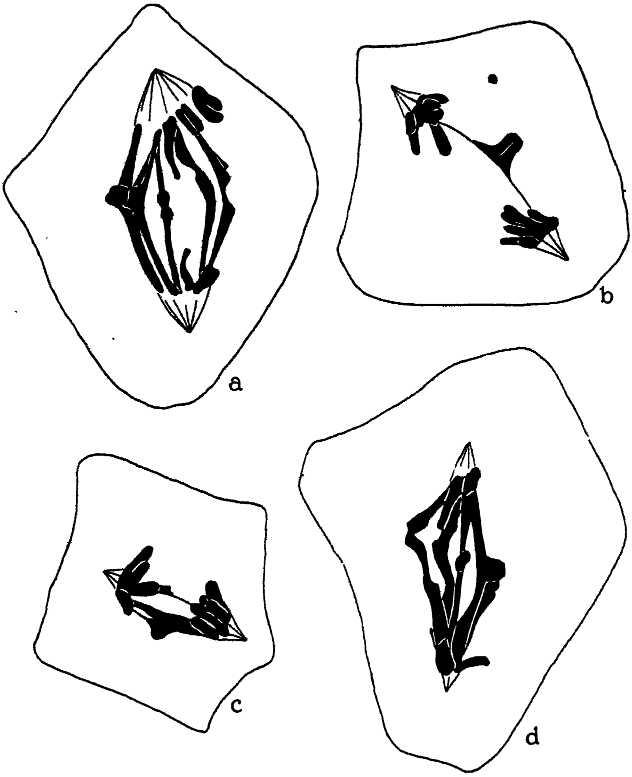

Fig. 4—*a*, *b*, *c* and *d* = anaphases of the first meiotic division in irradiated *Mecostethus*; 7¼ hr. material. All these anaphases have extensive "bridges" caused by the stickiness of the chromosomes.

When we turn to the material fixed 25 hr. after irradiation we find that the nuclei of the spermatocytes are still normal up to the end of the prophase of the first meiotic division (i.e. up to the end of diakinesis). From prometaphase onwards, however, a very marked change makes its appearance

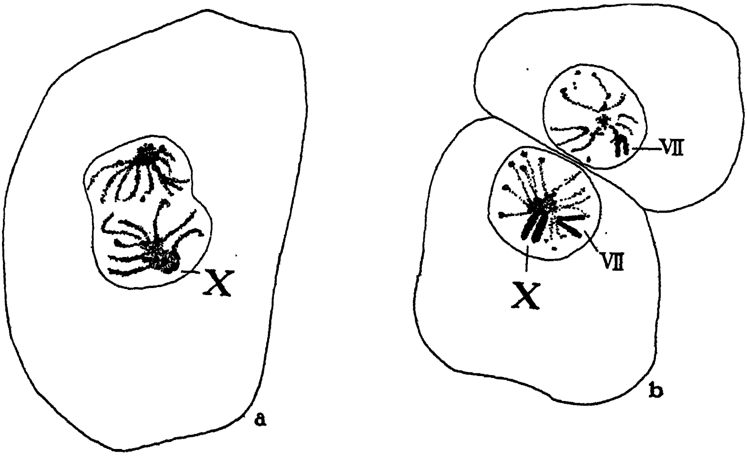

FIG. 5—Interkinesis in irradiated *Mecostethus*; 7½ hr. material. *a* = a "restitution nucleus" resulting from failure of separation of the two anaphase groups of chromosomes at the first meiotic division, *b* = two second spermatocyte nuclei (+*X* and −*X*).

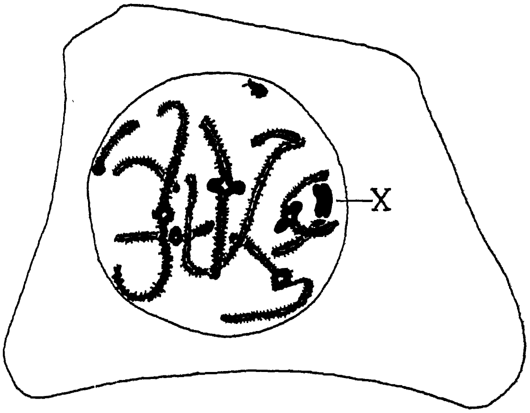

FIG. 6—Diakinesis in irradiated *Mecostethus*; 25 hr. material. Chromosomes normal and not fragmented.

and at the metaphase of the first division all the chromosomes have undergone a complete disintegration (fig. 7) so that they no longer bear the slightest resemblance to the normal set of bivalents at this stage (fig. 1*b*). This change is remarkably rapid and may best be understood by comparing the four figures 1*a*, 1*b*, 6 and 7*a*. The disintegration of the chromosomes illustrated in fig. 7 may probably be described by saying that the alteration in physical consistency already seen in the 7¼ hr. material has in the 25 hr. material gone so far that it has led to a complete liquifaction and fragmentation of all the chromosomes. It is interesting to note that the phenomenon does not begin until the nuclear membrane has disappeared and the chromosomes have become included in the spindle. Thus although it is obvious that there are many fragments or drops of liquid chromatin

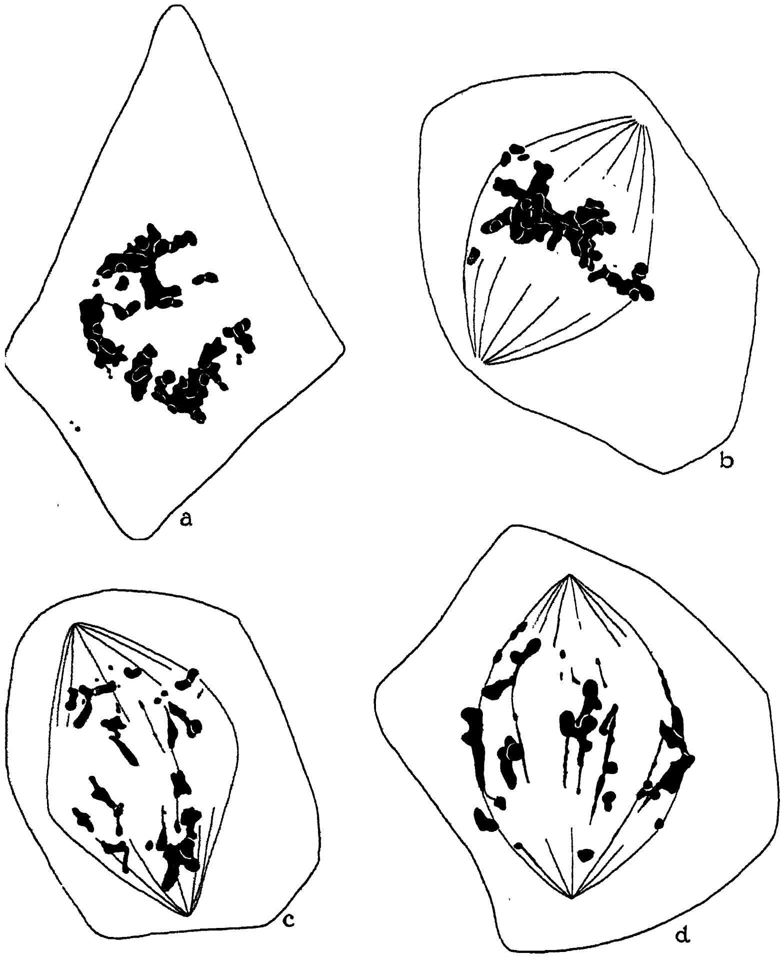

FIG. 7—*a* and *b* = polar and side view of first metaphase in irradiated *Mecostethus*; 25 hr. material. *c* and *d* = side views of anaphases in the same material.

which have no connexion with spindle attachments they are nevertheless included in the substance of the spindle (in fig. 7*a* there are two small fragments which are not in the spindle, but this is unusual). Figs. 7*a* and *b*, together with fig. 14, Plate 9, give a fairly good idea of the appearance of these completely disintegrated metaphases as seen in both side-views and polar view. The anaphases which result from them are illustrated in fig. 7*c* and *d*. Here long strings and drops of chromatin are drawn out in the spindle so that the whole appearance is quite irregular. There is some suggestion in these figures that the spindle-attachments are still functional, but one cannot be certain. There is no longer, as in the 7¼ hr. material, any impediment to anaphase-separation; the chromosomes are too disintegrated, and probably also too liquid to oppose any resistance to the elongation of the spindle. Consequently the interkinesis nuclei which result from these anaphases in the 25 hr. material are not restitution nuclei; they show a number of granules of chromatin, but no recognizable chromosomes.

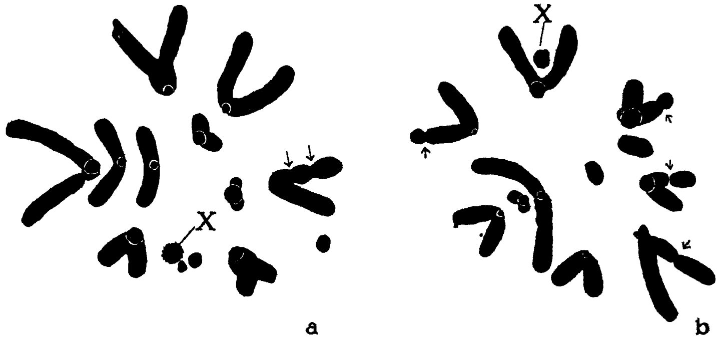

FIG. 8—*a* and *b* = metaphases of the first meiotic division in irradiated *Mecostethus*; 72 hr. material. The bivalents are mostly normal, except for the presence of a number of breaks (indicated by arrows).

The final fate of these cells has not been followed out in detail, but it is clear that they will not give rise to sperms capable of fertilizing an egg.

In material fixed 72 hr. after irradiation the complete disintegration of the chromosomes observed in the 25 hr. material is no longer found. The metaphases (fig. 8) are approximately normal but there are frequently present constrictions or actual gaps in the chromosomes which probably represent points of breakage, either of one or both chromatids. The frequency of occurrence of these breaks will form the subject of a subsequent paper, and figs. 8*a* and *b* are merely reproduced here in order to show that the "general disintegration effect" is a transitory one which disappears before the end of the third day after irradiation.

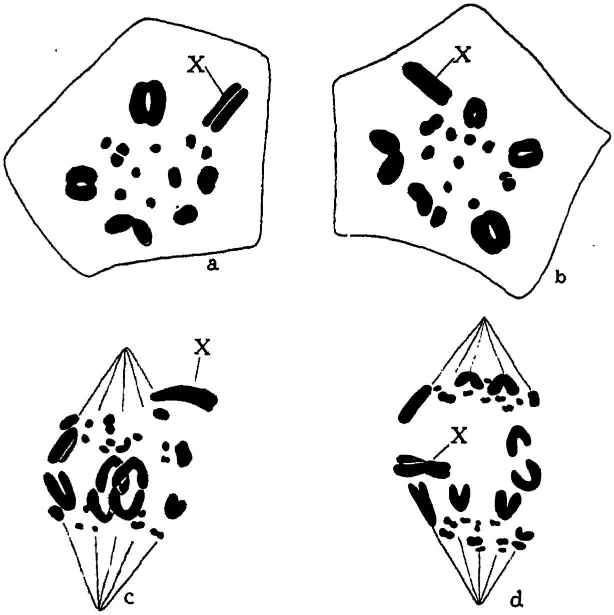

FIG. 9—Normal meiosis in *Metrioptera*. *a* and *b* = metaphases, *c* and *d* = anaphases of the first meiotic division.

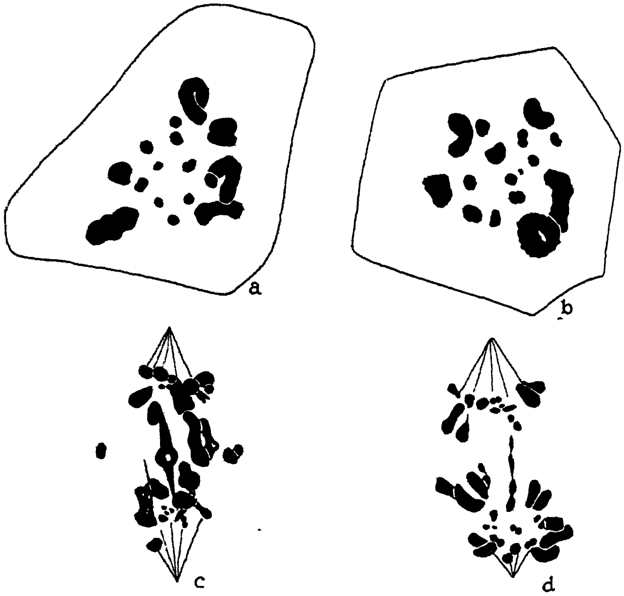

FIG. 10—Meiosis in irradiated *Metrioptera*; 25 hr. material. *a* and *b* = metaphases, *c* and *d* = anaphases of the first meiotic division.

5—MEIOSIS IN X-RAYED *METRIOPTERA BRACHYPTERA* AND *STAURODERUS BICOLOR*

The effects of irradiation appear to be very similar in *Stauroderus* to those found in *Mecostethus*. Fig. 11 and fig. 15, Plate 9, illustrate the complete disintegration of the first metaphases 22 hr. after irradiation. The effects of the two dosages (5 min. and 20 min.) are not very markedly different, although the disintegration is somewhat greater in the material irradiated for 20 min.

In *Metrioptera brachyptera* on the other hand, the effect is not nearly so profound, either in the 7¼ hr. or the 25 hr. material. In the 25 hr. material (fig. 10) the outlines of the metaphase chromosomes are somewhat irregular and they have some difficulty in separating at anaphase, but there is nothing like the complete disintegration seen in *Mecostethus*. It is worth noting that the material of these two species was directly comparable since they were irradiated simultaneously. As a matter of fact the anaphases in the 25 hr. material of *Metrioptera* were fairly similar to those in the 7¼ hr. material of *Mecostethus*. Unfortunately no 72 hr. material of *Metrioptera* was fixed, as the number of individuals available was too small. It is consequently not possible to say whether *Metrioptera* is more resistant to X-rays than *Mecostethus*, or whether the effect develops later. It is also not known whether a higher dosage would produce a real disintegration of the chromosomes of *Metrioptera*.

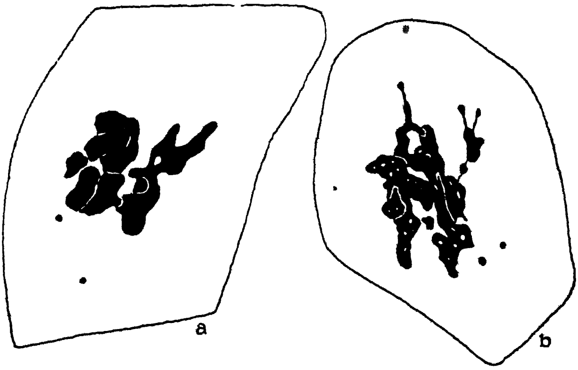

FIG. 11—Metaphases of first meiotic division in irradiated *Stauroderus*. *a* = in material irradiated for 5 min., *b* = in material irradiated for 20 min. Both fixed 22 hr. after irradiation.

6—DISCUSSION

Various authors (Politzer 1934; Stone 1933; Mather 1934) have described phenomena which present a certain similarity to those described above. Stone and Mather found a certain number of metaphases and anaphases which showed a great deal of "fragmentation". They assumed that this was of the same kind which leads to inversions, translocations, etc.; but it is more probable that they were dealing with a general disintegration of the chromosomes of the same type that I have described in *Mecostethus* and *Stauroderus*. Mather's figs. 12–14 in particular resemble my figures fairly closely. Mather put forward an elaborate theory to account for the "fragmentation" he observed in *Vicia* and *Tradescantia*; he suggested that in *Tradescantia* the great degree of "fragmentation" was due to the terminalization of the chiasmata which was in part responsible for breakage. It is clear that this hypothesis is not applicable to the three species of Orthoptera I have described here, since in none of them is there any appreciable amount of terminalization. Politzer (1934) has described pycnotic metaphases and anaphases which result from irradiation. It is not clear what relation exists between pycnosis and the phenomenon of chromosome disintegration; possibly they are only quantitatively different, but it seems more likely that they are really distinct phenomena.

When one attempts to analyse the way in which irradiation causes the phenomenon of chromosome-disintegration, one fact stands out as significant; the chromosomes are not affected during prophase, i.e. so long as they are surrounded by the nuclear membrane. This applies not only to the autosomes but also to the X (see fig. 6) which has reached the metaphase degree of contraction half-way through prophase (i.e. in diplotene). This suggests that the nuclear membrane acts in a protective way, isolating the chromosomes from direct contact with some substance or substances which are present in the cytoplasm of the irradiated cell. If this is so it is clear that the substance, whatever its nature, is not produced immediately after irradiation but develops gradually, so that in *Mecostethus* there is relatively little of it present 7¼ hr. later, considerably more than 25 hr. after irradiation. The substance is eventually (3 days after irradiation) either destroyed or in some other way prevented from acting.

The hypothesis of a substance or substances, probably enzymatic in nature, which directly attacks the chromosomes in such a way as first of all to alter their physical consistency, and finally to lead to their complete disintegration appears to be the most satisfactory way of explaining the effects which are described above. As to how the immediate ionization which results from irradiation produces the substance, whether by actually synthesizing it, by liberating it as a result of a destruction of the cytoplasmic structure, or merely by destroying another inhibitor substance—all these are mere speculations. But that the "disintegration effect" is due to some chemical agency in the cytoplasm which cannot penetrate the nuclear membrane appears highly probable. If this is so it means that the "disintegration effect" is entirely different from the ordinary chromosome-breakage which has hitherto been studied by geneticists. It may, however, account for some of the sterility observed in irradiated genetical material. On the hypothesis presented above one would not expect to find the "disintegration effect" in non-dividing somatic cells (where the chromosomes are protected by the nuclear membrane) or in sperms (where there is practically no cytoplasm). It is clear that the effect also varies from species to species in extent, as can be seen by comparing the results on *Mecostethus* with those on *Metrioptera*.

7—SUMMARY

Irradiation of spermatocytes in three species of Orthoptera leads to profound changes in the chromosomes at the first meiotic division. These changes do not manifest themselves until some hours after irradiation. The first change is an alteration of the physical consistency of the chromosomes which tends to prevent their separation at anaphase. Later on this change in the physical state of the chromosomes leads to their complete disintegration. It is suggested that the phenomenon is entirely different from ordinary chromosome "fragmentation" (as seen in inversions, translocations, etc.), and that it results from the destructive action of a substance produced or liberated in the cytoplasm as a result of irradiation.

REFERENCES

Darlington, C. D. 1936 *J. Genet.* **33**, 465–500.
Helwig, E. R. 1933 *J. Morph.* **55**, 265–312.
Huskins, C. L. and Hunter, A. S. W. 1935 *Proc. Roy. Soc.* B, **119**, 22–33.
Janssens, F. A. 1909 *Cellule*, **25**, 387–411.
— 1924 *Cellule*, **34**, 135–359.
Mather, K. 1934 *Hereditas, Lund*, **20**, 303–22.
McClung, C. E. 1927 *J. Morph.* **43**, 181–264.
— 1928 *Arch. Biol., Paris*, **38**, 503–28.
Politzer, B. 1934 "Pathologie der Mitose." Berlin: Borntraeger.
Riley, H. P. 1936 *Cytologia, Tokyo*, **7**, 131–42.
Stone, L. H. A. 1933 *Ann. Bot., Lond.*, **41**, 815–26.
White, M. J. D. 1933 *Cytologia, Tokyo*, **5**, 135–9.
— 1935 *Proc. Roy. Soc.* B, **119**, 61–84.
— 1936 *Z. Zellforsch.* **24**, 128–35.

Description of Plate 9

FIG. 12—Anaphases of the first meiotic division in *Mecostethus grossus*; 7¼ hr. material.

FIG. 13—The same.

FIG. 14—Metaphases of the first meiotic division in *Mecostethus grossus*, showing complete disintegration of the chromosomes; 25 hr. material.

FIG. 15—Metaphases and anaphases of the first meiotic division in *Stauroderus bicolor* irradiated for 20 min. and fixed 22 hr. later.

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The Effect of Androgenic Compounds on the Histological Structure of the Pituitary in the Castrated Rat

By Marjorie Allanson

Department of Zoology, King's College, London

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I—Introduction

It is known that the administration of certain hormone preparations will prevent the development of the characteristic histological changes in the anterior pituitary of the castrated male rat. This has been effected by implants of testicular and ovarian tissue, injection of testis extracts and of female hormones; the literature has been adequately reviewed by Severinghaus, Engle and Smith (1932) and Nelson and Gallagher (1936). Some of the crystalline male hormone preparations now available have also been used with success. Schoeller, Dohrn and Hohlweg (1936) compared the amounts of testosterone, testosterone propionate, androsterone and oestradiol benzoate required to prevent the appearance of changes for 15 days after castration in immature and adult rats of both sexes. They found that in the adult male a daily dose of $0 \cdot 15\gamma$ of oestradiol benzoate, 150γ of testosterone propionate, 300γ of testosterone, and 2000γ of androsterone was successful. In the adult female more oestradiol benzoate is necessary ($0{\cdot}3\gamma$), but less androsterone (1500γ): the doses of testosterone and testosterone propionate are the same for the two sexes. In immature animals (29–33 g. body weight), the doses necessary are considerably smaller. Wolfe and Hamilton (1937), using a standard dose of 500γ daily for 10 days, found that testosterone, testosterone propionate and testosterone acetate maintained the normal pituitary structure in immature males, and that testosterone acetate and oestrone were successful in immature females.

Another type of experiment, the restoration of the pituitary to normal in animals castrated for several weeks, has been carried out by several investigators. Ovarian and testicular implants and injections of testis extracts and female hormones have been used with varying success. Korenchevsky, Dennison and Simpson (1935) report that androsterone, androstanediol and oestrone will restore the hypertrophied pituitary of the castrated rat to the normal weight, and cause partial restoration of the histological structure. With testosterone and testosterone propionate there was no definite return to normal weight (Korenchevsky, Dennison and Eldridge 1937).

In the present paper the power of thirteen crystalline male hormone preparations to correct the post-castration changes in the pituitary is compared with that of oestrone and crude testis extracts. The glands were obtained from animals used by Deanesly and Parkes in their work on the biological properties of compounds of the androsteronetestosterone series (Deanesly and Parkes 1936*a*, *c*, *d*, 1937*a*). They have kindly supplied the data on the response of the accessory organs, so that a comparison can be made between the activity of the male hormones in correcting the post-castration changes in the anterior pituitary and in the accessory organs. It is evident from the literature that oestrin and related compounds are capable of maintaining and of restoring the anterior pituitary of both sexes after castration, and it is possible that the effect of male hormone compounds is due to their oestrogenic rather than to their androgenic activity. The pituitary response has therefore been studied from the standpoint of the oestrogenic as well as of the androgenic properties of the injected compounds.

II—MATERIAL AND METHODS

A list of the hormone preparations used is given in Table I, together with details of administration and duration of treatment. The experimental technique has been described by Deanesly and Parkes (1936*a*, *c*).

Animals—Rats were castrated at 40–60 g. body weight (in a few experiments at 170–200 g. body weight) and used not less than a month later. Neither the weight of the rat nor the time after castration when treated were standardized, but control animals of appropriate weight and length of castration were obtained for all experiments. In the maintenance experiments, treatment was started on the day of castration.

The control animals numbered fifteen normal males of 60–280 g. body weight, and twenty-four untreated castrates killed 14–136 days after operation.

The prostate and seminal vesicles were weighed after fixation.

Administration of Hormone Preparations—Hormones were administered in arachis oil, nut oil or propylene glycol. In the preliminary experiments the volume of oil was kept as low as possible, but it was later found that greater effect on the accessory organs was obtained by increasing the volume of oil, and after the experiments of Miescher, Wettstein and Tschopp (1936*a*, *b*) further tests were carried out with the addition of palmitic acid to the oil solution, and with the use of esters. More recently it was found that administration of certain of the free hormones in the solid condition by a single subcutaneous implantation is even more effective than the addition of palmitic acid to the oil solution or the esterification of the hormone. Experiments on the administration of the hormones in these different ways have provided a series of animals in which varied effects have been produced on the accessory organs by the same total dose of hormone, and this has made it possible to compare the effectiveness of restoration of the pituitary and of the accessory organs.

Histological Technique—The animals were killed with chloroform and the pituitary dissected out and fixed within 5–10 min. Sousa's fixative followed by a modification of Mallory's triple stain was used as a routine method, as the results are dependable and the castration cells stain brilliantly. Since this technique does not differentiate the unmodified basophil cells satisfactorily, pituitaries from representative control and experimental animals were fixed and stained by the methods of Wolfe and Cleveland (1932), Severinghaus (1932) and Crooke and Russell (1935).

The glands were sectioned serially at 4μ in the horizontal plane, every 25th section being mounted and examined. No attempt has been made to do cell counts. The proportional changes in the cell types were sufficiently marked to make this unnecessary, and all diagnoses were checked by comparison of sections by a Reichert comparimeter eyepiece. With this double eyepiece two slides can be examined simultaneously, and striking differences in cell size, affinity for stains, and proportions of cell types, can readily be distinguished.

III—HISTOLOGY OF THE ANTERIOR PITUITARY AFTER CASTRATION

The progressive changes in histological structure following castration have been described in detail by Ellison and Wolfe (1934) in the female rat; by Nukariya (1926) and others in the male. Nukariya found that there is no marked difference in the structure of pituitaries of rats castrated prepubertally and those castrated when adult. The castrate controls provide a series of glands from 14 to 136 days after castration, and the various stages were found to agree closely with those described by previous authors. In the normal male gland the number of basophil cells is insignificant. According to Wolfe and Phelps (1935) they represent 6·5% of the total cells; Nelson (1935) places them at 8·1%. Large granular basophils are fairly plentiful at the junction of the intermediate and anterior lobes: at the periphery and in the central region they are fewer and occur singly. Within 14 days after castration the number of large basophils is markedly increased at the expense of the chromophobes, they are abundant at the periphery and are more common in the centre. They continue to increase in number and size, and by 30 days after operation they are the most prominent cell type in all parts of the anterior pituitary. The number of these swollen cells remains high throughout castration, although there is a slight proportional decrease after 90 days. The so-called "signet-ring" or castration cells appear early, but are not present in appreciable numbers until about 30 days after operation. They are at first small basophil cells with a colloid vacuole about the size of the nucleus, and are found at the junction of the intermediate and anterior lobes. Later they are distributed round the periphery, and finally throughout the gland. By 60 days after operation the majority of these cells measure 25 by 25μ, and are mostly occupied by colloid. Their number rises rapidly until about 90 days after castration, and Ellison and Wolfe (1934) found in the female rat that it continues to increase, but at a slower rate, until 180 days. Nukariya (1926) and Severinghaus (1933) have reported that the eosinophils decrease in size, but measurement of these cells in normals and castrates has failed to show any size change in the present series of glands.

TABLE I

Compound	No. of rats	Days after castration at autopsy	Total dose mg.	Medium	Days treated	No. of administrations	Average wt. of prostate and seminal vesicles mg.	Response of pituitary
Saturated compounds:								
Androsterone	11	73–124	10·0	Propylene glycol, nut oil, or arachis oil	10	10	134	0
Androstanediol	5	56	2·5	5·0 c.c. arachis oil	10	10	123	0
,,	5	112	5·0	2·0 c.c. arachis oil	10	10	157	0
,,	5	56	10·0	2·0 c.c. arachis oil	10	10	194	0
,,	4	112	1·5	—	20	1	316	+ to + +
Androstanedione	5	60	10·0	10·0 c.c. arachis oil	20	20	243	+ +
Unsaturated compounds:								
*Trans*dehydroandrosterone	5	60	10·0	10·0 c.c. arachis oil	20	20	—	0 to +
*Trans*androstenediol	5	60	10·0	10·0 c.c. arachis oil	20	20	—	+
,,	5	85	10·0	2·0 c.c. arachis oil	10	10	204	0
,,	5	85	5·0	2·0 c.c. arachis oil	10	10	105	0
Androstenedione	2	68	5·0	2·0 c.c. arachis oil	10	10	160	+
,,	5	68	10·0	2·0 c.c. arachis oil	10	10	186	+ + +
,,	5	60	10·0	10·0 c.c. arachis oil	20	20	714	+ + + +
Testosterone	5	92	2·0	2·0 c.c. arachis oil	10	10	88	0
,,	5	67	4·0	2·0 c.c. arachis oil	10	10	143	+
,,	5	67	6·0	2·0 c.c. arachis oil	10	10	221	+
,,	5	67	8·0	2·0 c.c. arachis oil	10	10	255	+ to + +
Testosterone with 0·5 g. palmitic acid	10	49	2·0	5·0 c.c. nut oil	10	10	435	+ + +
Testosterone	5	60	10·0	10·0 c.c. arachis oil	20	20	1105	+ + + +
,,	3	96	3·3	—	10	1	828	+ to + +
,,	8	82	2·8	—	20	1	541	+ + +

Esters:								
Testosterone acetate	2	78	2·29	5·0 c.c. arachis oil	10	10	427	+ + to + + +
,, ,,	5	78	2·29	2·0 c.c. arachis oil	20	10	290	+ +
Testosterone acetate with 0·5 g. palmitic acid	3	78	2·29	5·0 c.c. arachis oil	10	10	393	+ +
Testosterone propionate	5	60	10·0	10·0 c.c. arachis oil	20	20	1563	+ + + +
,, ,,	4	106	2·1	—	10	1	441	+ + +
,, ,,	5	112	2·74	—	20	1	803	+ + +
Methylated compounds:								
Methylandrostanediol	5	60	7·5	2·0 c.c. arachis oil	10	10	279	+
Methyldihydrotestosterone	4	136	7·5	2·0 c.c. arachis oil	10	10	366	0 to +
Methyltestosterone	5	65	2·0	2·0 c.c. arachis oil	10	10	107	0
Testis extracts:								
Bull testis	4	106	1 c.c.	4·0 c.c. nut oil	10	10	202	0
,,	1	78	2 c.c.	2·0 c.c. nut oil	10	10	282	0 to +
,,	1	78	4 c.c.	4·0 c.c. nut oil	10	10	463	+ + +
Boar testis	5	60	2 g.	5·0 c.c. nut oil	20	20	—	+
Oestrone	2	78	0·5 mg.	5·0 c.c. arachis oil	10	10	—	+ + +
,,	5	60	1·0	10·0 c.c. arachis oil	20	20	—	+ + + +
Maintenance experiments:								
Androstanediol	5	14	4·7	—	14	1	714	+ + + +
,,	5	30	6·7	—	30	1	1291	+ + + +
Testosterone	2	14	1·4	—	14	1	1399	+ + + +

IV—HISTOLOGY OF THE ANTERIOR PITUITARY OF THE CASTRATED RAT AFTER TREATMENT

(*a*) *Restoration to Normal Structure*—The various compounds were not of equal potency in causing reversal of the castration changes, and as some compounds were tested over 10- and 20-day periods, all stages in restoration to the normal pituitary structure could be studied. The earliest change is a gradual decrease in the number of enlarged basophil cells. They disappear first from the centre of the gland, and later from the periphery; the relative numbers of basophil and chromophobe cells return to normal. This is followed by decrease in size and reduction in numbers of the castration cells. As with the enlarged basophils, the changes start in the central region so that it is possible to find a gland with the central area normal in structure, but with numerous large castration cells at the periphery. Complete removal of the castration cells was not effected in 20 days with any compound used, but, since Halpern and D'Amour (1936) found them present in female rats injected with large doses of oestrin over 8 weeks, it is evident that these cells disappear very slowly. A pituitary has, therefore, been judged to have been successfully restored when the structure is normal except for occasional small castration cells.

As the enlarged basophils are almost completely absent before any decrease in size or number of the castration cells is apparent, the pituitary during restoration presents a histological picture unlike any stage in the development of post-castration changes.

In Tables I and II the following symbols have been used to indicate the degree of restoration present:

+ + + + histological structure normal except for the presence of occasional small castration cells;

+ + + large castration cells numerous at periphery but not prominent in the centre, large basophils not more numerous than in normal gland;

+ + no change in size and number of castration cells but a marked decrease in number of enlarged basophils;

+ enlarged basophils disappearing from centre, no change in castration cells; *or* much variation in structure in different parts of the gland.

(*b*) *Maintenance of Normal Structure*—The pituitaries of animals implanted with free hormone at the time of castration and killed 14 and 30 days later showed none of the post-castration changes typical for these stages. A few small cells with colloid vacuoles are present in the glands

TABLE II

Compound	No. of rats	Days after castration at autopsy	Total dose	Days injected	Average wt. of prostate and seminal vesicles mg.	Response of pituitary
Oestrone	5	60	1·0 mg. in 10 c.c. arachis oil	20	—	+ + + +
Boar testis extract	5	60	2·0 g. in 5·5 c.c. oil	20	—	+
*Trans*androstenediol	5	60	10·0 mg. in 10·0 c.c. arachis oil	20	—	+
*Trans*dehydroandrosterone	5	60	,,	20	—	0 to +
Androstenedione	5	60	,,	20	714	+ + + +
Testosterone	5	60	,,	20	1105	+ + + +
Testosterone propionate	5	60	,,	20	1563	+ + + +
Androstanedione	5	60	,,	20	243	+ +

of normal rats, and in the "maintained" pituitaries they are about twice as numerous as in the normal. Many experimental treatments, involving the introduction of foreign proteins to the blood stream, will bring about a small increase in the number of these cells (McEuen, Selye and Thomson 1934). Their presence has therefore not been judged significant, since the swollen basophils typical of the early castrate are absent.

V—RESTORATION EXPERIMENTS

(*a*) *Androsterone*—Pituitaries were obtained from four different experiments with androsterone (see Table I). In each case the total amount of androsterone was 10 mg. administered in daily injections over 10 days. The pituitaries showed no significant change from the castrate structure.

The largest accessory glands in this series occurred in animals receiving androsterone in propylene glycol, the weights being: prostate, 169 mg., seminal vesicles, 34 mg.

(*b*) *Androstanediol* was administered in arachis oil to three groups of five rats each, in doses of 2·5, 5·0 and 10·0 mg. The largest dose produced fair development of the accessory organs, particularly of the seminal vesicles, but failed to effect any restoration of the pituitary.

Another experiment, in which the androstanediol was administered as a subcutaneously implanted pellet (average dose 1·5 mg.) and the rats killed 20 days later, resulted in partial restoration of the pituitary and in much increased growth of the seminal vesicles and prostate.

(*c*) *Androstanedione*—One experiment on a group of five rats was carried out with this compound, 10 mg. being given over 20 days in a total of 10 c.c. oil. The pituitaries showed partial restoration. Greater growth of the prostate was obtained than with ten daily injections of androsterone or androstanediol, but there was little growth of the seminal vesicles.

(*d*) *Transdehydroandrosterone*—Five rats were given 10 mg. in arachis oil over 20 days, with negative results on the accessory organs. In the pituitary, however, there were indications that correction of the castrate structure had commenced.

(*e*) *Transandrostenediol*—In two 10-day experiments a total of 5 and 10 mg. of the compound was administered in 2 c.c. oil. The larger dose gave a fair growth of the accessory organs, particularly of the seminal vesicles, and the pituitary showed signs of restoration. In one 20-day experiment with a total dose of 10 mg. in 10 c.c. oil, the accessory organs gave no response, but pituitary restoration had commenced. In this case, the ineffectiveness of the compound on the accessory organs is almost certainly due to the large volume of oil used.

(*f*) *Androstenedione*—Three groups of rats received androstenedione: 5 mg. over 10 days, 10 mg. over 10 days, and 10 mg. over 20 days. All these showed some restoration of the pituitary, and, in the 20-day experiment, the restoration to normal was almost complete. Good results were obtained with the accessory organs.

(*g*) *Testosterone*—Experiments with testosterone in oil solution, in oil solution with palmitic acid, with testosterone in tablet form, with testosterone acetate, and with testosterone propionate in oil solution and in tablet form, were carried out. The results recorded in Table I give clear evidence of correlation between the effect on the accessory glands and on the pituitary. Even large doses of the free hormone given in concentrated oil solution failed to produce more than the initial stages of pituitary restoration, and were relatively ineffective on the accessory glands. Almost complete restoration was, however, obtained with a large dose of the free hormone given in a large volume of oil over 20 days, correlated with very good growth of the accessory glands.

A small dose of testosterone with palmitic acid gave moderate restoration of both accessory glands and pituitary, while the same amount of testosterone in oil without the addition of palmitic acid resulted in poor response of the accessory organs and of the pituitary.

The three rats receiving one implant of free testosterone in tablet form (approximately 2 mg.) and killed 10 days later, showed better growth of the accessory organs compared with the rats receiving the same amount of hormone with palmitic acid, but restoration of the pituitary was not so advanced. The reason for the poor response of the pituitary in this experiment is not obvious. Five rats receiving practically the same amount of free hormone in the same manner and killed 20 days later, showed greater restoration of the pituitary, but the accessory glands had apparently passed their maximum development and were smaller than those found in the 10-day experiment.

(*h*) *Testosterone acetate*—Pituitaries were available from three experiments with this highly effective ester. The results varied from partial to half-complete restoration of the pituitary, in keeping with the growth of the accessory organs.

(*i*) *Testosterone propionate*—Daily administration of 0·5 mg. of testosterone propionate in 0·5 c.c. oil over 20 days gave the largest accessory glands observed in this series of experiments. Maximum restoration of the pituitary was found.

Tablets of testosterone propionate varying from 1·0 mg. to 3·0 mg. were implanted into eight rats, three animals being killed at 10 days and five at 20 days after implantation. A better response of both accessory glands and pituitary was obtained from the 20-day experiment, but was not as great as obtained by daily injections.

(*j*) *Methylandrostanediol*—Five rats were given 7·5 mg. of this compound in oil, over 10 days. The results on both accessory organs and pituitary were slightly better than with the non-methylated substance.

(*k*) *Methyldihydrotestosterone*—A dose of 7·5 mg. in oil over 10 days gave a moderate recovery of the accessory organs. In the pituitary there were indications that restoration had commenced.

(*l*) *Methyltestosterone*—Five rats received 2·0 mg. in oil over 10 days. The results on the accessory organs were poor, and the pituitary showed no signs of restoration.

(*m*) *Testis extracts*—Since no male hormone compound had effected complete restoration in 10 days, experiments were made with a few animals to find the degree of correction produced by testis extracts over the same period. A dose of 1 c.c. of bull testis extract in 4·0 c.c. nut oil failed to alter the castrate structure of the pituitary, but had some effect on the accessory organs; 2·0 c.c. in 2·0 c.c. oil gave a slight response in the pituitary, and 4·0 c.c. in 4·0 c.c. oil caused greater growth of the accessory organs and a degree of restoration of the pituitary comparable with results obtained in 10-day tests with 10 mg. of androstenedione and 2 mg. of testosterone with palmitic acid. A heavy dose of boar testis extract (2·0 g. in 5·0 c.c. oil over 20 days) produced no growth in the accessory organs and little correction in the pituitary.

(*n*) *Oestrone*—Several investigators report complete restoration of the pituitary of the castrated male rat by injection of oestrin and other female hormone preparations (Montpellier and Chiapponi 1930; Nelson 1934; Crooke and Korenchevsky 1935), but most of the experiments described were of more than 20 days duration. Experiments were accordingly carried out with oestrone to find the correction produced in 10-day and 20-day periods, for comparison with the results obtained with male hormone preparations. Two groups of rats were given doses of 0·5 mg. of oestrone in 5·0 c.c. arachis oil over 10 days, and 1·0 mg. in 10·0 c.c. oil over 20 days. Correction of the pituitary was no further advanced than in tests with the most effective male hormones.

VI—Maintenance Experiments

(*a*) *Androstanediol*—Five animals were implanted at the time of castration with approximately 4·7 mg. of the free hormone and killed 14 days later. The accessory organs were slightly smaller than those of control normal animals, but in the pituitary, castration changes were absent. In another group of five rats implanted with approximately 6·7 mg. and killed 30 days later, the accessory organs were larger than in the control animals and the pituitary structure was normal. It is of interest to compare these results with the restoration experiments with this compound which suggest that it possesses little androgenic power.

(*b*) *Testosterone*—Implants of approximately 1·4 mg. prevented the appearance of castration changes in the pituitary. The weight of the accessory organs was more than 50 % above that of the controls.

VII—Relation between Response of the Pituitary and the Oestrogenic Properties of Male Hormones

Some of the experiments already described were planned to test whether the response of the anterior pituitary was related to the oestrogenic power of the male hormones. The compounds and extract used, arranged in descending order of oestrogenic activity (Deanesly and Parkes 1936*b*, 1937*b*), are listed in Table II. This seriation is similar to that given by Korenchevsky, Dennison and Eldridge (1937), except for the position of testosterone. A uniform dose of 10·0 mg. in 10·0 c.c. arachis oil of each of the male hormones was administered over 20 days to six groups of five rats. Two other groups received 1·0 mg. of oestrone in 10 c.c. oil and 2 g. of boar testis extract in 5·5 c.c. oil respectively. The weights of the animals and the time since castration were constant for all experiments. The boar testis extract was selected as being comparatively highly oestrogenic, but little androgenic. It is possible that the oestrogenic power of the male compounds has some influence on the response of the pituitary, since androstenedione has a greater effect than would be expected from the response of the accessory organs. The evidence from *trans*dehydroandrosterone and the boar testis extract is inconclusive, but comparison of the much more oestrogenic *trans*androstenediol with testosterone does not suggest any direct relation between oestrogenicity and power to restore the pituitary gland of the castrate. A much more regular relation is found between the response of the pituitary and the androgenicity of the compound as shown by the response of the accessory organs.

VIII—RELATION BETWEEN RESPONSE OF THE PITUITARY AND THE ANDROGENIC ACTIVITY OF MALE HORMONES

The experiments with testosterone, testosterone acetate, testosterone propionate, androstanediol, androstenedione, androstanedione and bull testis extract, provide sufficient data for an examination of the correlation between the response of the pituitary and androgenic activity. In fig. 1 the symbols denoting the degree of pituitary restoration are plotted against the combined weight of prostate and seminal vesicles. It will be seen that little restoration occurs when the accessory glands weigh less than 400 mg., except in the case of androstenedione, and that the pituitary is most completely restored when there is maximum growth of the accessory glands (800–1600 mg.). The results from the implantation of testosterone tablets for 10 days fall seriously below the general curve, since the accessory glands weigh over 800 mg. and in the pituitary there is little restoration. With this one exception, the response of the pituitary is related to that of the accessory organs fairly closely.

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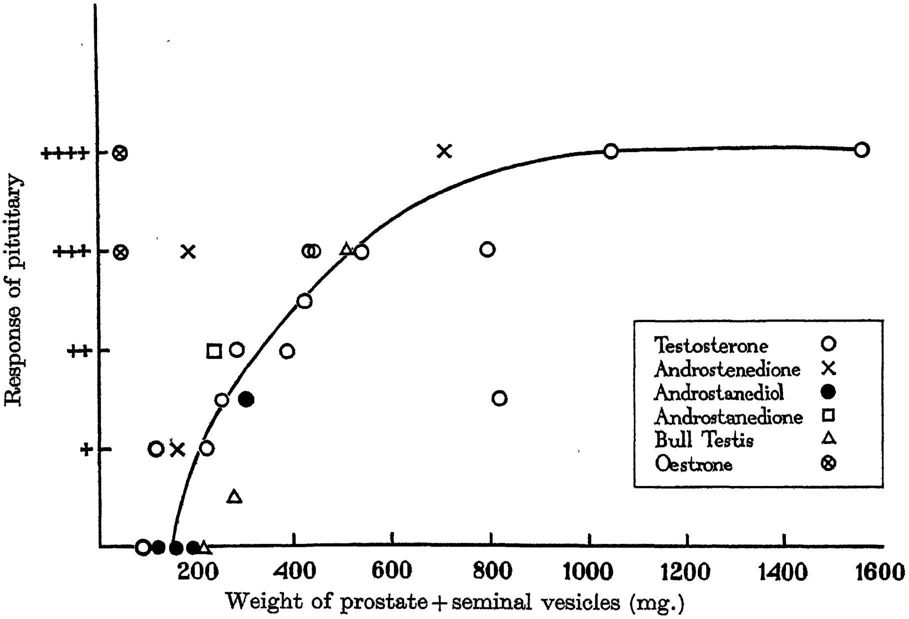

Fig. 1—Activities of various compounds on the anterior pituitary in relation to their activities on the accessory organs.

IX—Summary

1. The activity of thirteen male hormone compounds in restoring the pituitary of castrated adult rats to the normal structure has been compared. Hormones were administered by daily injections in oil, in oil with the addition of palmitic acid, or by a single subcutaneous implantation of a solid tablet.

2. Experiments on the restoration of the pituitary to normal were carried out for 10- and 20-day periods. Almost complete correction of the

pituitary was effected by twenty daily injections of androstenedione, testosterone and testosterone propionate.

3. The histology of the anterior pituitary during the reversal of the castration changes is described.

4. Little relation between the oestrogenic properties of male hormones and their power to restore the pituitary was found.

5. The response of the pituitary is correlated with the response of the accessory organs.

6. Subcutaneous implantations of androstanediol and testosterone will maintain the normal structure of the anterior pituitary for as long as 30 days.

REFERENCES

Crooke, A. C. and Korenchevsky, V. 1935 *Proc. R. Soc. Med.* **28**, 1266–7.
Crooke, A. C. and Russell, D. S. 1935 *J. Path. Bact.* **40**, 255–83.
Deanesly, R. and Parkes, A. S. 1936*a* *Biochem. J.* **30**, 291–303.
— — 1936*b* *Brit. Med. J.* **1**, 257–8.
— — 1936*c* *Brit. Med. J.* **1**, 527–8.
— — 1936*d* *Lancet* (i), 837–9.
— — 1937*a* *Chem. Ind.* **56**, 447.
— — 1937*b* *Quart. J. Exp. Physiol.* **26**, 393–402.
Ellison, E. T. and Wolfe, J. M. 1934 *Endocrinology*, **18**, 555–75.
Halpern, S. R. and D'Amour, F. E. 1936 *Amer. J. Physiol.* **115**, 229–38.
Korenchevsky, V., Dennison, M. and Eldridge, M. 1937 *Biochem. J.* **31**, 475–85.
Korenchevsky, V., Dennison, M. and Simpson, S. L. 1935 *Biochem. J.* **29**, 2534–51.
McEuen, C. S., Selye, H. and Thomson, D. L. 1934 *Brit. J. Exp. Path.* **15**, 221–3.
Miescher, K., Wettstein, A. and Tschopp, E. 1936*a* *Chem. Ind.* **55**, 238.
— — — 1936*b* *Schweiz. med. Wschr.* **66**, 310–12.
Montpellier, J. and Chiapponi, L. 1930 *C.R. Soc. Biol., Paris*, **104**, 373–5.
Nelson, W. O. 1934 *Proc. Soc. Exp. Biol., N.Y.*, **32**, 452–4.
— 1935 *Endocrinology*, **19**, 187–98.
Nelson, W. O. and Gallagher, T. F. 1936 *Anat. Rec.* **64**, 129–45.
Nukariya, S. 1926 *Pflüg. Arch. ges. Physiol.* **214**, 697–720.
Schoeller, W., Dohrn, M. and Hohlweg, W. 1936 *Klin. Wschr.* **15**, 1907–8.
Severinghaus, A. E. 1932 *Anat. Rec.* **53**, 1–5.
— 1933 *Anat. Rec.* **57**, 149–75.
Severinghaus, A. E., Engle, E. T. and Smith, P. E. 1932 "Sex and Internal Secretions", 1st ed. Chap. XVII. London: Baillière, Tindall and Cox.
Wolfe, J. M. and Cleveland, R. 1932 *Anat. Rec.* **51**, 409–13.
Wolfe, J. M. and Hamilton, J. B. 1937 *Anat. Rec.* **67**, suppl. p. 55.
Wolfe, J. M. and Phelps, D. 1935 *Proc. Soc. Exp. Biol., N.Y.*, **32**, 1305–9.

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Initiation of the Propagated Disturbance

By W. A. H. Rushton

Research Fellow of Emmanuel College, Cambridge

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PART I—GENERAL THEORY

It has long been supposed that the mechanism of propagation is the stimulation of the inactive region just in front, by the advancing action-potential wave. Plausible as is this theory, it is only within the last year that evidence has been published which puts the matter beyond doubt.

Blair and Erlanger (1936*b*) obtained suggestive evidence by blocking a single impulse with calcium or anodal polarization. They found that this blocked impulse left a transitory state of enhanced conductivity which allowed a second impulse to be propagated. The nature of this enhancement was studied independently and in greater detail by Hodgkin (1937). He used cold or pressure to block the first impulse, and found that the lowering of threshold on the farther side of the block had the same spatial and temporal distribution as had the spread of the action current. Moreover, the action current spread had the same space-time distribution as that of a subthreshold current applied from an external circuit, and so arranged as to have the same time course as the action-potential wave.

Thus, whatever other factors may conceivably be involved, the action current certainly spreads forward into the inactive region of a nerve in the same way as does a current from the outside, and this spread will account for the lowering of threshold observed. But since this lowering may be as much as 90 % at some distance in front of the active region, there can be little doubt that close to the active region itself, the lowering will be far more than 100 %.

We may thus conclude that the action-potential wave normally propagated is of an intensity many times that sufficient to excite the neighbouring portion of nerve; it is quite a different matter, however, to suppose that this statement applies to the action-potential wave, as it is first initiated at the cathode of a stimulating circuit. For the normal propagated wave consists of an active region some centimetres in length, each element of which may be supposed to contribute (though not equally) to the excitation of the region in front of the wave. On the other hand, the excitation wave at the moment of its inception will occupy only a very small length, and be correspondingly feeble and might in fact be insufficient to propagate unless fostered by the continued application of the external stimulus.

Now the classical treatment of the excitation process does not take this into consideration at all. It has been customary to assume not only that when a certain membrane in the neighbourhood of the applied cathode is depolarized to a critical extent the action-potential wave arises, but that this wave from the start is of sufficient intensity to propagate. This last conclusion, however, has never been justified, it is inconsistent with the above simple concept of the flow of action current, and moreover, the expectations to which it leads differ in certain respects from those where the feebleness of the initial response is considered. It therefore appeared worth while to investigate theoretically this concept of the initiation of the propagated disturbance.

Now, though a number of conclusions follow readily from intuitive considerations, one cannot obtain even approximately quantitative results without a detailed mathematical investigation, and this has the disadvantage that unless the assumptions are greatly over-simplified, the resulting equations are (to me at least) insoluble.

It has therefore seemed best to attempt the investigation as follows. Assumptions are made, in many cases grossly over-simple, but such as will allow of a relatively easy mathematical treatment over a very wide range of possible stimuli. A graphical analysis is described which will permit those not familiar with the underlying equations to obtain solutions in a routine manner for any given temporal form of stimulus, so that it will be relatively easy to compare experimental results with the expectations of theory over a wide field, and then it will often be easy to see how such deviations as will be found may be due to the over-simplification of the assumptions. Finally, if there is a general confirmation of the present concept, it may be worth while to develop the theory upon more accurate assumptions, but with incomparably greater difficulty.

The present treatment will consist of three sections. In the first the assumptions will be stated and discussed, the second will contain the mathematical treatment, and the resulting conclusions will be summarized in the third. Those, therefore, who do not wish to examine the details of the mathematics may omit the middle section and still appreciate the assumptions and the conclusions.

In Part II, the general conclusions of the first part will be applied to a number of specific cases to show that the present theory fits in a fairly satisfactory manner a wide range of known observation, and to point out a number of conditions where certain results should be expected which differ from the classical expectations. Mention will also be made of the principal observations which will not fit the present theory.

ASSUMPTIONS

The nerve is supposed to have a cable structure, the axis cylinder and the interstitial fluid being relatively well conducting, and the myelin sheath being highly resistant. These resistances are supposed to be non-capacitative. The nodes of Ranvier are neglected. Closely applied to the myelin sheath is a fine membrane which is supposed to be capacitative, but so leaky that its resistance is negligible compared with that of the myelin sheath which is in series with it.

Each element of inactive nerve sheath may be represented by the circuit (A), fig. 1, where R is the ohmic resistance of the myelin, c the capacity of the fine membrane applied closely to it, ρ the leak resistance of the membrane, where ρ/R is negligible. Finally E is the resting potential across the sheath which is observed as the injury potential.

It is clear that the resting charge on the condenser will be altered by passage of current through the myelin, and we assume that when this alteration attains a critical value, the local condition of the membrane suddenly changes. The intimate nature of this change is not considered, but the result is that the resting potential E is abolished, so that the sheath in the active region is represented by (B), fig. 1.

Since any given element of sheath is connected with all other elements through the conducting interstitial fluid and axis cylinder, it follows that the change of potential arising where the given element changes from inactive to active state, will affect in some degree all points on the nerve, though principally the regions adjacent. The nature of this spread is illustrated in fig. 2.

Suppose that the whole nerve is inactive except for a stretch 5 mm. in length whose centre point is O. Then the potential of each point on the surface of the nerve due simply to the activity of this stretch is given by the lowest member of the family of continuous curves in fig. 2. The other members of the family correspond, in order, to the potential distribution when the active lengths are 10, 20, 30, 40, 50 mm.

It is seen that each curve is concave towards the horizontal axis in the central region, and convex on either side. The transition is at an abscissa corresponding exactly to the edge of the active region. The dotted curve passes through these inflexion points, hence the horizontal distance between the two intersections for any member of the family gives the corresponding length of active nerve.

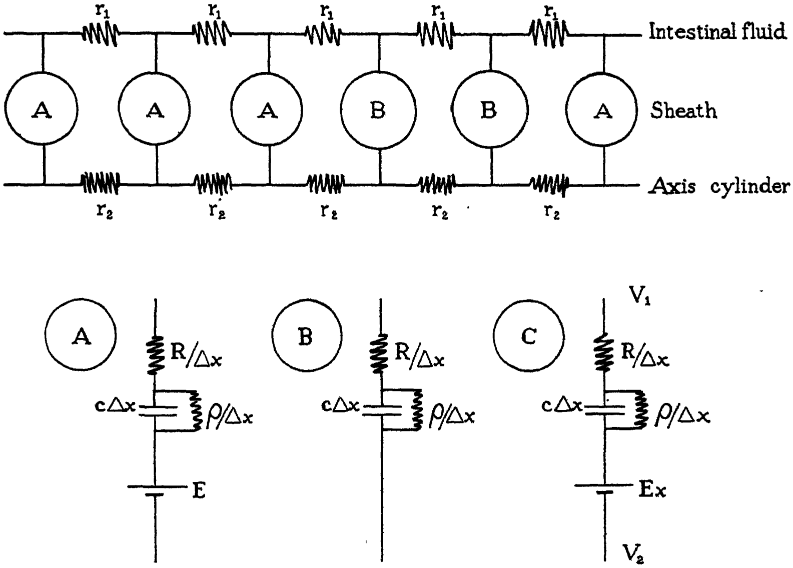

FIG. 1—Schematic circuit diagram of nerve.

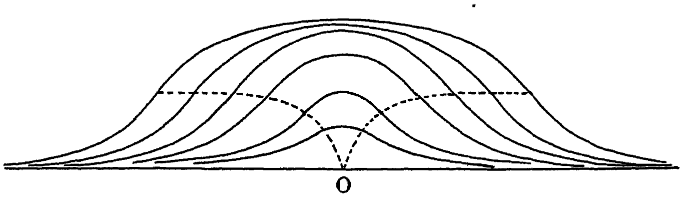

FIG. 2—Abscissae: distances along nerve. Ordinates: potential of surface of nerve due to the activity of various lengths. *O* is the centre of the active stretch, and for each curve, the limits of the active region are the abscissae corresponding to the intersection of the curve with the dotted exponentials.

From fig. 2 it is easily appreciated that 5 mm. of active nerve only develops about one-quarter the action potential of a long active stretch, comparing the potentials at *O*, the mid-points of the two active regions;

and about one-half comparing the junctions of active and inactive regions in the two cases.

Shorter active lengths will clearly develop smaller potentials as given by the corresponding values of the dotted curve.

MATHEMATICAL SECTION

Let

V_1 = potential outside the sheath.
V_2 = potential inside.
i_1 = current flowing parallel to nerve outside.
i_2 = current flowing parallel inside.
$I = i_1 + i_2$.
r_1 = resistance per unit length of interstitial fluid.
r_2 = resistance per unit length of axis cylinder.
$1/R$ = conductivity per unit length of sheath for current passing radially.
$1/\rho$ = conductivity per unit length of membrane.
θ = charge per unit length on membrane.
θ_1 = critical value of θ at which nerve becomes active.
E = potential in series with sheath responsible for injury and action potential.
c = capacity of membrane per unit length.
$L^2 = R/(r_1 + r_2)$.
$\alpha = c\rho$ = finite time.
$\rho/R \rightarrow 0$.

CURRENT DISTRIBUTION IN NERVE

The nerve is represented electrically by the scheme of fig. 1, where the elements are supposed to be infinitesimal and infinitely numerous. The element of sheath is represented either by (A) or (B), according as it is inactive or active at that point.

Currents from external circuits are led into the interstitial fluid according to any known time-space distribution, and it is required to find an expression for the charge on the membrane at any point and any time as a combined result of nerve activity and external stimulus.

To obviate the necessity of going over the working separately for the two types (A) and (B), let us assume that the sheath has the more general electrical structure (C), where E_x may be either E or zero as particular cases.

Then, applying Kirchoff's Laws to (C),

$$\begin{aligned} &\text{Since current through condenser element} &&= \dot{\theta}\Delta x, \\ &\text{and current through shunt element} &&= \theta\Delta x/c\rho, \\ \therefore\ &\text{current through } R/\Delta x &&= \left(\dot{\theta}+\frac{\theta}{c\rho}\right)\Delta x, \end{aligned}$$

$$\therefore \qquad V_1-V_2 = R\left(\dot{\theta}+\frac{\theta}{c\rho}\right)+\frac{\theta}{c}+E_x.$$

But from the main network we have

$$-\frac{\partial V_1}{\partial x} = r_1 i_1, \quad -\frac{\partial V_2}{\partial x} = r_2 i_2, \quad i_1+i_2 = I.$$

Also $$\frac{\partial i_2}{\partial x} = \text{current density through sheath } R$$

$$= \left(\dot{\theta}+\frac{\theta}{c\rho}\right).$$

$$\therefore \quad \frac{\partial}{\partial x}\left[R\left(\dot{\theta}+\frac{\theta}{c\rho}\right)+\frac{\theta}{c}+E_x\right] = \frac{\partial}{\partial x}(V_1-V_2)$$

$$= r_2 i_2 - r_1(I-i_2).$$

$$\therefore \quad \frac{\partial^2}{\partial x^2}\left[R\left(\dot{\theta}+\frac{\theta}{c\rho}\right)+\frac{\theta}{c}+E_x\right] = (r_1+r_2)\left(\dot{\theta}+\frac{\theta}{c\rho}\right)-r_1\frac{\partial I}{\partial x}.$$

Now putting $$c\rho = \alpha, \quad R/(r_1+r_2) = L^2,$$

$$\frac{\partial^2}{\partial x^2}\left(\dot{\theta}+\frac{\theta}{\alpha}\right)-\frac{1}{L^2}\left(\dot{\theta}+\frac{\theta}{\alpha}\right) = -\frac{\theta}{\alpha}.\frac{\rho}{R}-\frac{r_1}{R}.\frac{\partial I}{\partial x}-\frac{\partial^2 E_x}{R\partial x^2},$$

and since $\rho/R \to 0$,

$$\left(\frac{\partial^2}{\partial x^2}-\frac{1}{L^2}\right)\left(\dot{\theta}+\frac{\theta}{\alpha}\right) = -\frac{r_1}{R}.\frac{\partial I}{\partial x}-\frac{\partial^2 E_x}{R\partial x^2}.$$

To solve, we note that if

$$\left(\frac{L^2\partial^2}{\partial x^2}-1\right)y = u,$$

$$2yL = e^{-\frac{x}{L}}\int_{-\infty}^{x} e^{\frac{x}{L}}u\,dx + e^{\frac{x}{L}}\int_{x}^{\infty} e^{-\frac{x}{L}}u\,dx$$

is a solution, as may be verified by differentiation.

Thus putting
$$y=\left(\dot{\theta}+\frac{\theta}{\alpha}\right)\Big/L^2,$$
$$u=-\frac{r_1}{R}\cdot\frac{\partial I}{\partial x}-\frac{\partial^2 E_x}{\partial x^2},$$
we obtain
$$-\frac{2R}{L}\left(\dot{\theta}+\frac{\theta}{\alpha}\right)=e^{-\frac{x}{L}}\int_{-\infty}^{x}e^{\frac{x}{L}}\left(r_1\frac{\partial I}{\partial x}+\frac{\partial^2 E_x}{\partial x^2}\right)dx+e^{\frac{x}{L}}\int_{x}^{\infty}e^{-\frac{x}{L}}\left(r_1\frac{\partial I}{\partial x}+\frac{\partial^2 E_x}{\partial x^2}\right)dx. \quad (1)$$

That this solution is the particular one to suit the boundary conditions is seen by putting $x=\pm\infty$, when the second member of equation (1) vanishes, provided that $\partial I/\partial x$ and $\partial^2 E_x/\partial x^2$ are both zero for points far from the origin.

Since E_x is essentially a discontinuous function, being either E or zero, exception might be taken to the form $\partial^2 E_x/\partial x^2$ in (1), and the same in lesser degree applies to $\partial I/\partial x$. Since, however, equation (1) applies to any continuous function E_x, it will apply to $1+\tanh qx$, which can be made to differ by less than any assignable quantity from the discontinuous function with which we have to deal, by making q large enough. Hence (1) applies to the discontinuous case with infinitesimal error.

Equation (1) may be put into a slightly more convenient form by integrating by parts the quantity $\partial^2 E_x/\partial x^2$, giving
$$-\frac{2R}{L}\left(\dot{\theta}+\frac{\theta}{\alpha}\right)=r_1\left\{e^{-\frac{x}{L}}\int_{-\infty}^{x}e^{\frac{x}{L}}\frac{\partial I}{\partial x}dx+e^{\frac{x}{L}}\int_{x}^{\infty}e^{-\frac{x}{L}}\frac{\partial I}{\partial x}dx\right\}$$
$$+\frac{1}{L}\left\{-e^{-\frac{x}{L}}\int_{-\infty}^{x}e^{\frac{x}{L}}\frac{\partial E_x}{\partial x}dx+e^{\frac{x}{L}}\int_{x}^{\infty}e^{-\frac{x}{L}}\frac{\partial E_x}{\partial x}dx\right\}, \quad (2)$$
where the coefficient of r_1 gives the contribution from the applied stimulus, and the coefficient of $1/L$ the contribution from the active portions of nerve.

It would be easy, integrating by parts again, to change the quantities under the integral sign from $\partial I/\partial x$, $\partial E_x/\partial x$ to I and E_x, but the above form is more convenient. For I and E_x in the following analysis will be constant over a certain range and then jump suddenly to a different constant value. This allows the integral in the form (2) to be written down at once. For, throughout the range in which I is constant, $\partial I/\partial x$ is zero and contributes nothing. If at x_n, I changes from I_n to I_{n+1}, then the value of
$$\int_{x_n-\Delta x}^{x_n+\Delta x}e^{-\frac{x}{L}}\frac{\partial I}{\partial x}dx=e^{-\frac{x_n}{L}}\int_{x_n-\Delta x}^{x_n+\Delta x}dI=e^{-\frac{x_n}{L}}(I_{n+1}-I_n).$$
The full value of the integral is thus simply
$$\sum^{n}e^{-\frac{x_n}{L}}(I_{n+1}-I_n). \quad (3)$$

BIPOLAR ELECTRODES

Suppose that the cathode is at $x = 0$, and the anode at $x = -s$. Then I vanishes except for values of x between the electrodes, since in the extrapolar regions all the current flowing away in the core must return in the interstitial fluid, and hence $i_1 + i_2 = I = 0$. Between the electrodes $I = I_1$, the current passed from the external circuit. These assumptions apply fairly accurately to the usual conditions of excitation. With regard to the potential E_x, we assume that until θ attains a value θ_1 it is E. But as soon as θ_1 has been attained E_x immediately assumes a fixed value zero which is maintained indefinitely.

At face value this is a poor approximation to the truth, since the action-potential wave appears to increase rather linearly than abruptly (Erlanger and Blair 1933), and certainly lasts 1 or 2 msecs. But it must be remembered that it has not been possible to record the onset of the action-potential wave in circumstances uncomplicated by propagation and capacitative spread, and all we know is that at the seat of generation of the action potential the time course must certainly be more rapid than any recorded wave.

With regard to the assumption of persistence of the action potential, this is chiefly erroneous for slowly rising stimuli where accommodation enters and complicates the matter in any case.

Suppose that at any moment t, the excited region extends from $-X_1$ to X_2, where X_2 is to the right of the cathode and $-X_1$ to the left. Consider the value of θ at a point x to the right of X_2.

Then in equation (2) the integrals from x to ∞ vanish, and applying formula (3) we immediately obtain

$$\frac{2R}{L}\left(\frac{\partial\theta}{\partial t}+\frac{\theta}{\alpha}\right) = e^{-\frac{x}{L}}(1-e^{-\frac{s}{L}})\,I_1 r_1 + e^{-\frac{x}{L}}(e^{\frac{X_2}{L}} - e^{-\frac{X_1}{L}})\frac{E}{L}. \tag{4}$$

Now, if we make the substitution

$$\theta = \phi e^{-\frac{x}{L}}$$

in (4) and divide throughout by $e^{-\frac{x}{L}}$ we obtain

$$\frac{2R}{L}\left(\frac{\partial\phi}{\partial t}+\frac{\phi}{\alpha}\right) = (1-e^{-\frac{s}{L}})\,I_1 r_1 + (e^{\frac{X_2}{L}} - e^{\frac{X_1}{L}})\frac{E}{L},$$

which shows that ϕ is independent of x.

But we know that at $x = X_2$, $\theta = \theta_1$, thus at any greater value of x

$$\theta = \theta_1 e^{-\frac{x-X_2}{L}}. \tag{5}$$

Thus in the extrapolar region the charge diminishes exponentially with the distance away from the nearest point of the active region with length constant L. We may therefore learn whether the active region is advancing or not by finding whether the charge at some fixed point in front is increasing. This is obtained by combining (4) and (5), with the elimination of θ,

$$\frac{2R}{E}\cdot\frac{\partial\theta}{\partial t}\cdot e^{\frac{x}{L}} = -\frac{2R\theta_1}{\alpha E}e^{\frac{X_2}{L}} + \frac{LI_1r_1}{E}(1-e^{-\frac{s}{L}}) + e^{\frac{X_2}{L}} - e^{-\frac{X_1}{L}}, \tag{6}$$

from which we see that the second member must be positive if propagation is to occur at the instant considered.

Liminal Length—From equation (6) it is clear that the stimulus I_1 to be effective must produce a change of such a magnitude that when the stimulus is withdrawn $\partial\theta/\partial t$ will still be positive.

$$\therefore\quad (e^{\frac{X_2}{L}} - e^{-\frac{X_1}{L}}) > \frac{2R\theta_1}{\alpha E}e^{\frac{X_2}{L}}.$$

$$\therefore\quad X_2 + X_1 > -L\log_e\left(1 - \frac{2R\theta_1}{\alpha E}\right). \tag{7}$$

Now the second member is independent of the nature of the stimulus. Hence a necessary condition for stimulation is that a liminal length of nerve $X_2 + X_1$, defined as above, be excited by the time that the stimulus ceases.

Put
$$1 - \frac{2R\theta_1}{\alpha E} = h = e^{-\frac{X_2+X_1}{L}} \tag{8}$$

at the liminal length. The constant h, which has no dimensions, plays an important part in all the present treatment and may be called the "propagation constant" of the tissue.

This condition of a liminal length has just been shown to be a necessary one, and it is easily seen to be sufficient. For, once the propagation has begun after cessation of the stimulus, the only change in conditions is that a greater and greater length of nerve becomes active, and hence a greater and greater stimulus is applied. The condition of the liminal length assures an initial velocity of propagation, positive though infinitesimal, and as a greater length becomes active the velocity will increase to a fixed value.

VELOCITY OF PROPAGATION AND SAFETY FACTOR

At a great distance from the origin, equation (6) becomes

$$\frac{2R}{E}\cdot\frac{\partial\theta}{\partial t} = -\frac{2R\theta_1}{\alpha E}e^{\frac{X_2-x}{L}} + e^{\frac{X_2-x}{L}},$$

or, combining with (5) and (8),

$$\frac{\partial\theta}{\partial t} = \frac{h\theta}{\alpha(1-h)}.$$

But since in this region the impulse is travelling with constant velocity v, we have

$$\frac{\partial\theta}{\partial t} = -v\frac{\partial\theta}{\partial x} = \frac{v\theta}{L}.$$

Hence
$$v = \frac{Lh}{\alpha(1-h)}. \tag{9}$$

Now the safety factor may be defined as one less than the ratio of the existing value of E to the value which E must assume to reduce the velocity just to zero. But from (8) it is seen that E is inversely as $(1-h)$. Hence the safety factor is one less than the reciprocal of the ratio of the existing value of $(1-h)$ to the value that this must assume to abolish conduction velocity. But from (9) the latter condition is satisfied by $h = 0$ or $1-h = 1$.

Hence
$$\text{Safety factor} = \frac{h}{1-h}. \tag{10}$$

THE RHEOBASE

The rheobase must have the intensity to raise to the value θ_1 the charge immediately under the cathode, and it must furthermore be sufficient to allow propagation from this point indefinitely. We proceed to show that if the first condition is satisfied, the second will also be satisfied.

Let I_0 be the value of I_1 which satisfies the first condition, then from (4) we see that I_0 must have such a value that $\partial\theta/\partial t$ vanishes when

$$\theta = \theta_1 e^{-\frac{x}{L}},$$

X_2 and X_1 being zero. Thus

$$\frac{I_0 r_1 L}{E}(1-e^{-\frac{s}{L}}) = \frac{2R\theta_1}{\alpha E} = 1-h. \tag{11}$$

Putting $I = I_0$, equation (6) becomes

$$\frac{2R}{E}\cdot\frac{\partial\theta}{\partial t}\cdot e^{\frac{x}{L}} = -(1-h)(e^{\frac{X_2}{L}}-1)+e^{\frac{X_2}{L}}-e^{-\frac{X_1}{L}}$$

$$= h(e^{\frac{X_2}{L}}-1)+(1-e^{-\frac{X_1}{L}}).$$

And since $X_2 > 0 > -X_1$, $\partial\theta/\partial t$ is always positive and I_0, the least current which will activate the cathode, will propagate indefinitely, and is hence the rheobase.

Excitation with Short Interpolar Length

We are now in a position to consider approximately the conditions of efficacy of a stimulus of any known time course. But since this efficacy depends upon the way in which propagation is initiated from the cathode, it will be convenient to consider specifically two cases of bipolar electrodes, namely, where the two electrodes are very close together, or very far apart, since in these two cases the problem is fairly simple.

When the electrodes are very close together, the excitation spreads at first entirely into the extrapolar region. For clearly the stimulus has no exciting effect at the point midway between the electrodes (which is very close to the cathode in the case considered), hence this point will not be raised to activity until there is already more than the liminal length of nerve excited in the extrapolar region. Thus the applied stimulus, by the time that it ceases, must have raised to activity the region from the origin to a point X, where from equation (8)

$$e^{-\frac{X}{L}} = h.$$

Consider then the value of θ at this point X. The condition that a stimulus may be adequate is that at the moment of its cessation θ has attained a value of at least θ_1. A stimulus is threshold when any diminution in strength abolishes the foregoing condition.*

Considering this value of θ in equation (4) we obtain

$$\frac{2R}{hE}\left(\frac{\partial\theta}{\partial t}+\frac{\theta}{\alpha}\right) = \frac{I_1 r_1 s}{E}+e^{\frac{X_2}{L}}-1.$$

---

* It is not accurate to simplify the above statement and say that a threshold stimulus is one where $\theta = \theta_1$ at the cessation of the stimulus. Take, for example, a constant current which lasts for a time somewhat longer than the utilization time of the rheobase. If this current is below rheobasic intensity θ will never attain θ_1 (nor in fact $h\theta_1$). If on the other hand the current is just adequate, θ will attain θ_1 by the utilization period, and be much greater by the time that the current ceases.

Now there are two conditions in which X_2 is constant, i.e. in which the propagation is not advancing. The first is during that period when the charge under the cathode has not yet attained the value θ_1, in which case $X_2 = 0$, and the equation reduces to that for the charging of a shunted condenser. The second case is when, although X_2 has some positive value, yet, in the event of the removal or the diminution of the applied stimulus, the value of $\partial\theta/\partial t$ ceases to be positive. Since the active stretch continues to be active in such a case, X_2 remains constant, and propagation may be resumed if the stimulus increases again.

In both cases, therefore, we have an ordinary first order equation, which may be simplified by substitution from (8), and by introducing the rheobase value from (11), with s very small,

$$I_0 = \frac{E}{r_1 s}(1-h).$$

We thus obtain
$$\alpha\frac{\partial\theta}{\partial t}+\theta = h\theta_1\left(\frac{I_1}{I_0}+\frac{e^{\frac{X_2}{L}}-1}{1-h}\right). \tag{12}$$

Now, when X_2 is increasing, the value of θ at X_2 is θ_1, hence at X

$$\theta = \theta_1 e^{-\frac{X_2-X}{L}} = h\theta_1 e^{\frac{X_2}{L}},$$

from which we can substitute for X_2 in equation (12) and obtain

$$\alpha\frac{\partial\theta}{\partial t}+\theta = \frac{\theta_1 h}{1-h}\left[\frac{I_1}{I_0}(1-h)+\frac{\theta}{h\theta_1}-1\right],$$

or
$$\frac{\alpha(1-h)}{h}\cdot\frac{\partial\theta}{\partial t}-\theta = \theta_1\left[\frac{I_1}{I_0}(1-h)-1\right]. \tag{13}$$

In passing between the two ranges governed by equations (12) and (13) account must be taken of the fact that, so long as the stimulus remains of finite intensity, θ is continuous. Thus, initially θ is zero, it increases to the value $\theta_1 h$ where it leaves the first range of integration, and this value is consequently the initial value in the second range of integration.

At the moment when the impulse is initiated, θ changes from one range of integration to the other. The value of θ when this occurs may be called the "transitional value".

Again, from equation (12) it appears that, so long as I_1 suffers no discontinuity at the moment of changing range, the value of $\partial\theta/\partial t$ will also be continuous. For, at the instant of changing, the three variables θ, I_1 and X_2 are all continuous and hence the expression for $\partial\theta/\partial t$ is continuous.

Thus, given a stimulus of any time course, the effect produced is obtained first by the application of equation (12) with X_2 and the initial value of θ both put zero. When θ attains the transitional value $h\theta_1$ we pass to (13) maintaining continuity of θ. A further change back to (12) will have to be made if, through diminution of stimulus, θ ceases to increase. A simple graphical method of working out these results will be described later in this paper.

EXCITATION WITH GREAT INTERPOLAR LENGTH

In this case all the conditions of excitation are symmetrical on either side of the cathode, consequently propagation will spread equally in both directions, and we have

$$X_1 = X_2,$$

and hence the liminal length is excited when the impulse has travelled only half as far from the cathode as in the former case with short interpolar length. Thus if X is the distance from the cathode in the present case

$$e^{\frac{X}{L}} = \sqrt{h}.$$

Putting these values in equation (4), substituting from (8) and introducing from (11) the value of the rheobase I_0, with s infinity,

$$I_0 = \frac{E}{r_1 L}(1-h),$$

we obtain an equation analogous to (12) for the value of θ at X.

$$\alpha\frac{\partial\theta}{\partial t} + \theta = \theta_1\sqrt{h}\left[\frac{I_1}{I_0} + \frac{e^{\frac{X_2}{L}} - e^{-\frac{X_2}{L}}}{1-h}\right]. \tag{14}$$

Now when X_2 is increasing

$$\theta = \theta_1 e^{\frac{X_2}{L}}\sqrt{h}.$$

Hence
$$\alpha\frac{\partial\theta}{\partial t} = \theta_1\sqrt{h}\left[\frac{I_1}{I_0} + \frac{\sqrt{h}}{1-h}\left(\frac{\theta}{\theta_1} - \frac{\theta_1}{\theta}\right)\right]. \tag{15}$$

Now θ/θ_1 in the range of equation (15) lies between $\sqrt{h}$ and unity, hence if h is not less than 0·75, we may with an error less than 2·5 % put

$$\frac{\theta_1\sqrt{h}}{\theta} = 2 - \frac{\theta}{\theta_1\sqrt{h}}.$$

Introducing this into equation (15) we obtain

$$\frac{\alpha(1-h)}{1+h}\cdot\frac{\partial\theta}{\partial t}-\theta=\frac{\theta_1\sqrt{h}}{1+h}\left[\frac{I_1}{I_0}(1-h)-2\right]. \tag{16}$$

SOLUTION BY GRAPHICAL ANALYSIS

In a recent publication (Rushton 1937) I have described how by means of a simple appliance made from a piece of card, and by a routine manipulation, it is easy to solve the equation

$$a\frac{\partial y}{\partial t}+y=f(t),$$

where a is a constant, and $f(t)$ any given function of t.

Now all the equations (12), (13), 14), (16) are of this type and can therefore be solved in this way, which is indeed usually the easiest treatment. Moreover, since the matter is a perfectly routine one, it may be carried out by those unfamiliar with the mathematics underlying the equations to be solved, and they can in this way readily obtain an accurate graphical solution for any given form of stimulus.

We shall first consider the case where the electrodes are close together, and describe the procedure of the analysis without digressing to justify the statements made. Then the modifications required for the case of great interpolar distances will be given, and finally the validity of the process will be demonstrated.

Two analysers are required, one with subtangent α, and the other $\alpha(1-h)/h$. We know the rheobase I_0 and the stimulus I_1 at any instant, hence we may plot I_1/I_0 on a convenient vertical scale against time measured horizontally, and so scaled that α occupies about 2·5 cm. An example is shown in fig. 3 where the stimulus is a current which increases exponentially from zero to a constant value, and is represented by the continuous curve I_1/I_0 passing through the origin.

Two horizontal lines are drawn, one dotted at ordinate value unity, and one continuous at $1/h$. The curve I_1/I_0 is now redrawn, "reflected" as it were in the dotted horizontal, but with a different ordinate scale. We shall refer to this curve as the "reflected curve". It may be obtained first by reflecting I_1/I_0 in the ordinary way, and then reducing the ordinate distances between the curve and the dotted horizontal in the ratio $(1-h)/h$, i.e. the ratio of the two subtangents to be used. The reflected curve thus represents the quantity

$$\left[1-(1-h)\frac{I_1}{I_0}\right]\frac{1}{h}.$$

Now using the analyser with subtangent α and starting at the origin, a curve is obtained which represents the development of θ at a point distant the liminal length from the cathode. Ordinates represent a linear scale in θ; the horizontal dotted line is $h\theta_1$, the transitional value of θ, and consequently is the level at which we change from equation (12) to (13); the upper horizontal line is θ_1, and indicates the earliest point at which the current may be cut off and still allow propagation. When the curve of θ reaches the dotted horizontal, the other analyser with subtangent $\alpha(1-h)/h$ is taken, and the curve continued without a break. This analyser must be used with the pivot to the left of the tracing pencil, since the subtangent in this case is negative; the curve to be analysed now is not I_1/I_0 but the reflected curve

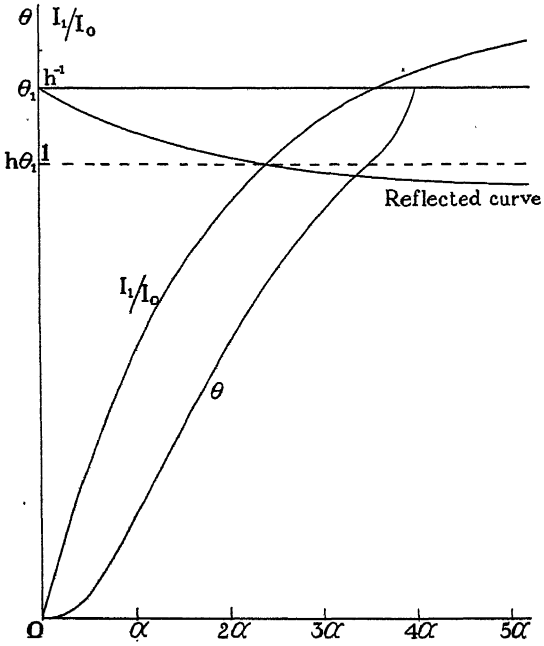

FIG. 3—Graphical analysis to obtain the curve of θ as function of time, given I_1, a stimulus of any known time course (see text).

$$\left[1-(1-h)\frac{I_1}{I_0}\right]\frac{1}{h}.$$

The curve of θ is seen to suffer no discontinuity of gradient (as already noted), but (in the present instance) to experience a point of inflexion at the transitional value. Though the impulse is initiated when the curve cuts the dotted horizontal, it is not until the upper horizontal is reached that the stimulus may be withdrawn without abolishing propagation.

If now the stimulating electrodes are far apart instead of being close together, some of the quantities in the foregoing analysis must be altered. The curve of I_1/I_0 is plotted as before, also the horizontal at ordinate unity, but this now corresponds to $\theta_1\sqrt{h}$, the transitional value for this case, hence the upper horizontal corresponding to θ_1 is drawn at ordinate $h^{-\frac{1}{2}}$. The first analyser is the same as before, but the second has a subtangent

$$\alpha\frac{1-h}{1+h}.$$

The reflected curve is drawn with distances from the dotted horizontal reduced in the ratio

$$\frac{1-h}{1+h},$$

which is the ratio of the subtangents in the present case, and therefore the curve represents the expression

$$\frac{2-(1-h)I_1/I_0}{1+h}.$$

As before, when the dotted horizontal is reached, the second analyser is used (pivot to the left), and the reflected curve is the one now treated. At the moment when θ meets the upper horizontal, the stimulus may be removed without abolishing conduction.

JUSTIFICATION

If in fig. 3 we adopt the scale which gives the value $\theta_1 h$ to the dotted horizontal, then the curve marked I_1/I_0 becomes the curve of $h\theta_1 I_1/I_0$, and this, analysed with subtangent α, gives a solution of θ which satisfies

$$\alpha\frac{\partial\theta}{\partial t}+\theta=h\theta_1\frac{I_1}{I_0},$$

which is equation (12) with X_2 zero.

The solution also satisfies the conditions that initially θ is zero and that it only applies to values below the transitional value $h\theta_1$. It is therefore the required solution.

In the same way the second range of the analysis satisfies equation (13), taking into account that the subtangent in this case is negative, being $-\alpha(1-h)/h$. Similarly the procedure for the case of great interpolar length satisfies the analogous equations (14) and (15).

TRIPOLAR ELECTRODES

If the current is led into the nerve by more than two electrodes, certain modifications must be made in some of the foregoing treatment, but in general the results are much the same, and do not merit a detailed consideration here. One case, however, is of particular interest, namely when the cathode of a tripolar system has two equal anodes symmetrically situated on either side of it, for unless s, the distance between two adjacent electrodes, exceeds a certain value, excitation is impossible however strong the stimulus.

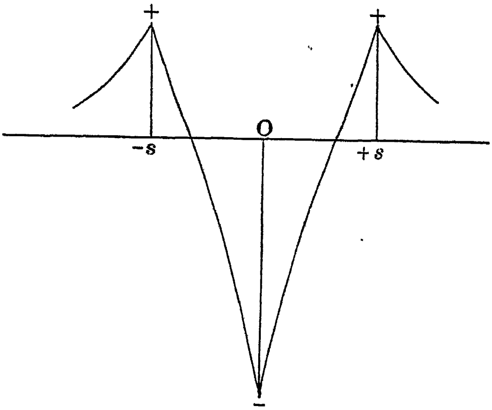

FIG. 4—Symmetrical tripolar electrodes. The density of current (ordinates) crossing the sheath is shown for each point on the nerve (abscissa).

Fig. 4 shows the charge produced at each point on the nerve between the points $-s$ and s by a current too weak to excite the cathode. It is easily seen from equations (2) and (3) that if at the cathode the charge is $2(1-e^{-\frac{s}{L}})$ then at any point x lying between 0 and s the charge θ_0 is given by

$$\theta_0 = 2e^{-\frac{x}{L}} - e^{-\frac{s-x}{L}} - e^{-\frac{s+x}{L}}, \tag{17}$$

hence the charge is zero when

$$e^{\frac{2x}{L}} = 2e^{\frac{s}{L}} - 1.$$

Suppose that the distance between the two points of zero charge is the liminal length, then

$$e^{-\frac{2x}{L}} = h$$

or

$$e^{-\frac{s}{L}} = \frac{2h}{1+h}. \tag{18}$$

Now it is clear that if s has a value less than this, propagation from the cathode will be impossible, for we have seen that a region no longer excited by external stimulus cannot be rendered active by less than the liminal length, and the region beyond the points of zero charge is negatively excited. Thus equation (18) represents the minimum value which s can have if any stimulus is to be effective. If s exceeds this value, however, we shall show that a rheobasic current merely sufficient to excite the cathodal point will propagate.

For from equation (4) we may write down the condition that charge shall increase at any instant, taking into account, that the contribution to excitation of the active region is independent of the nature of the electrodes but that the contribution of the applied stimulus is not the coefficient of I_1 in (4) but some multiple of θ_0 in (17).

We thus obtain

$$\frac{2R}{E}\left(\frac{\partial\theta}{\partial t}+\frac{\theta}{\alpha}\right)=e^{-\frac{x}{L}}\left(e^{\frac{X_2}{L}}-e^{-\frac{X_2}{L}}\right)+A\left\{2e^{-\frac{x}{L}}-e^{-\frac{s}{L}}\left(e^{-\frac{x}{L}}+e^{\frac{x}{L}}\right)\right\}.$$

Introducing the value that the current is rheobasic when $\partial\theta/\partial t$ vanishes for $\theta=\theta_1$ and $x=X_2=0$ we obtain

$$\frac{2R}{E}\frac{\partial\theta}{\partial t}+\frac{\theta}{\theta_1}(1-h)=e^{-\frac{x}{L}}\left(e^{\frac{X_2}{L}}-e^{-\frac{X_2}{L}}\right)+\frac{1-h}{2\left(1-e^{-\frac{s}{L}}\right)}\left\{2e^{-\frac{x}{L}}-e^{-\frac{s}{L}}\left(e^{-\frac{x}{L}}+e^{\frac{x}{L}}\right)\right\}.$$

Now introducing condition (18), and putting $x=X_2$, which also means $\theta=\theta_1$,

$$\begin{aligned}\frac{2R}{E}\frac{\partial\theta}{\partial t}&=-1+h+1-e^{-\frac{2X_2}{L}}+(1+h)e^{-\frac{X_2}{L}}-h\left(e^{-\frac{X_2}{L}}+e^{\frac{X_2}{L}}\right)\\&=e^{\frac{X_2}{L}}\left(1-e^{-\frac{X_2}{L}}\right)\left(e^{-\frac{2X_2}{L}}-h\right),\end{aligned}$$

and since $\quad 1>e^{-\frac{X_2}{L}}$ and $e^{-\frac{2X_2}{L}}>h,$

$\partial\theta/\partial t$ is always positive, and propagation will occur as far as the liminal length, but no farther. If, however, at this point the stimulating current ceases, propagation will continue indefinitely.

CONCLUSIONS FROM THE MATHEMATICS

1—In the extrapolar region the charge at the limit of the excited active portion has the critical value θ_1; the charge at other points at this instant diminish exponentially with their distance from this limit. The exponential constant is L, the constant of length for the cable (5).*

2—When an applied stimulus stops, the necessary and sufficient condition that propagation should continue is that a liminal length of nerve should have been excited. This length is given by expression (7).

3—A constant of no dimensions h is defined in (8) and called the "propagation constant". It appears in most of the mathematical expressions which have been derived.

4—It is clear that an adequate brief shock must excite the whole liminal length immediately. An enduring current on the other hand need only excite one point, whence the impulse, aided by the continued stimulus, will propagate indefinitely (11).

5—Expressions are derived for the final velocity of propagation (9) and for the safety factor (10).

6—Under the heading "Graphical Analysis" a method is described whereby the time course of excitation may be plotted in a routine fashion by those unfamiliar with differential equations, for the case of a stimulus of any given time course.

7—In the case of symmetrical tripolar electrodes, with the cathode in the middle, excitation is impossible unless the distance s between adjacent electrodes exceeds the value given by expression (18).

PART II—APPLICATION OF THE THEORY

1—Temporal Considerations

a—Brief Shocks

One of the chief difficulties in understanding the excitation process is that the only thing which can be observed about excitation itself, is that a given stimulus is or is not effective. All that we believe about the process is inference from the properties of various stimuli which either are or are not effective, together with a little collateral evidence derived from such physical and chemical concomitants as we imagine to be relevant.

In Part I of this paper a method has been described which will enable us to obtain a curve representing the way in which the excitation process is supposed to develop under the action of any given stimulus. At first sight it would seem that this whole curve is of no significance except as a means of determining the fact that in one case propagation will occur, and in another it will not; the intermediate part of the curve would seem to rest as highly speculative and be outside the range of experimental investigation. It becomes, therefore, a matter of the highest importance to the practical application of the theory that these curves may in fact be experimentally obtained by superposing brief shocks upon the stimulus to be analysed.

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* The numbers refer to the equations.

One Shock.

The action of a single instantaneous shock (hereafter called merely a "shock") is obviously to charge the nerve instantly to a value proportional to the strength of shock and distributed in space as is the coefficient of r_1 in equation (2). If θ is not raised to the critical value of θ_1 over a region equal to at least the liminal length, propagation cannot occur. A threshold shock will raise instantly to activity exactly this length. If we apply to a single shock the general graphical method described in Part I, it is clear that the effect of a threshold shock is to raise θ instantly to θ_1. Any other strength of shock will similarly increase θ by a value proportional to this strength.

Now turning to the more general case, we have seen that, from the given time curve of the stimulus, we can derive the curve of θ. We shall know θ at any instant if we know what we must add to θ to bring the value to θ_1. But it is just this information which is given by superposing a shock, and if at any instant this is done, and simultaneously the stimulus is cut off, it is clear that the threshold value of this shock is that necessary to increase θ to θ_1. By superposing threshold shocks, and simultaneously cutting off the stimulus at various instants, therefore, we may obtain experimentally a curve for comparison with that predicted.

Two Shocks.

The simplest case to consider is the curve due to one shock investigated by adding a second after a varying interval. Fig. 5 gives the results to be expected from the theory in the case where the electrodes are far apart. The curves fall into three classes. In the first the shock raises θ to an initial value θ_0 less than $\theta_1\sqrt{h}$, and the curve, after rising instantly, returns to zero along an exponential of subtangent α. In the second class, θ_0 has a value between $\theta_1\sqrt{h}$ and θ_1, which signifies that a region of nerve from $-X_2$ to $+X_2$ is made active where

$$e^{-\frac{X_2}{L}} = \sqrt{h}\,\theta_1/\theta_0.$$

The subsequent curve of θ is given by (14), where $I_1 = 0$, and

$$e^{-\frac{X_1}{L}} = e^{-\frac{X_2}{L}} = \sqrt{h}\,\theta_1/\theta_0.$$

$$\therefore\ \alpha\frac{\partial\theta}{\partial t}+\theta = \frac{\theta_1\sqrt{h}}{1-h}\left(\frac{\theta_0}{\theta_1\sqrt{h}}-\frac{\theta_1\sqrt{h}}{\theta_0}\right).$$

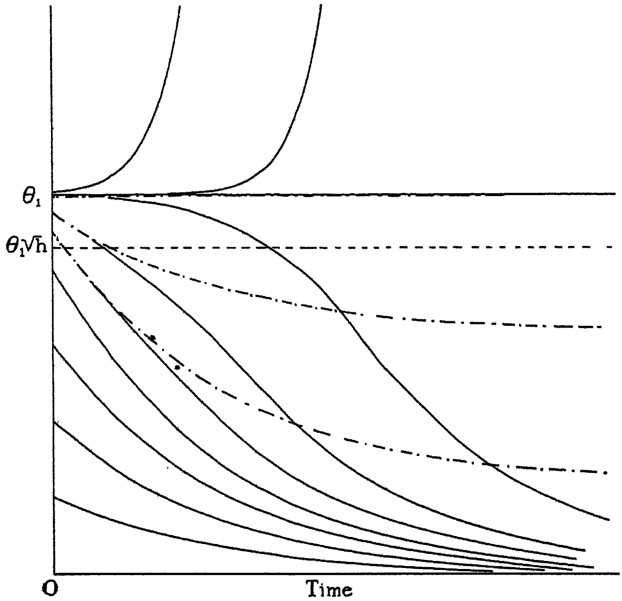

Fig. 5—The time course of θ following a single instantaneous shock applied at time O. The shape of the curve depends upon the strength of the shock (see text). For comparison with the experimental results of Katz (1937).

Since the second member of the equation does not vary with time, the curve of θ is an exponential of time constant α, as shown by the dotted curves in fig. 5. The initial value of each curve is θ_0, and the final value, obtained by putting $\partial\theta/\partial t = 0$, is

$$\theta_{t=\infty} = \frac{\theta_1}{1-h}\left(\frac{\theta_0}{\theta_1}-h\frac{\theta_1}{\theta_0}\right).$$

In particular it will be seen that when $\theta_0 = \theta_1$, both initial and final values are θ_1, and hence the curve coincides with the horizontal at that level.

Now the curves are calculated on the assumption that the action potential of the active stretch persists indefinitely, but we know that this is not the case, and that in fact the curves of θ instead of continuing horizontally at various levels must soon fall below these levels and return to the axis. The course then, that we should actually expect to find, is one which would coincide with the dotted curves of fig. 5 in their earlier course, and then fall to the axis as shown by the continuous curves in fig. 5. The third class of curves is when θ_0 is greater than θ_1, in which case propagation will occur unless an *anodic* shock is applied to reduce θ below θ_1 again. The curve in this case is obtained from the graphical analysis of the second range, which solves equation (16). The curves are shown in fig. 5, rising steeply above the level θ_1. They are exponentials asymptotic to the level θ_1, with subtangent $\alpha\frac{1-h}{1+h}$.

The special significance of these curves is that qualitatively at least they resemble very closely those recently obtained by Katz (1937) in his experimental investigation of this relation. His results show curves of the three classes mentioned above, each exhibiting the appropriate characteristics.

It may be of interest to remark that the present theory and Katz's experiments were independent. Hodgkin and I had worked out these expectations, and sketched some experimental details of the modification considered below, when Katz came to discuss with us his first evidence of deviation from the simple exponential decay.

Three Shocks

If a shock is quickly followed by one that is equal but in the opposite direction, the subsequent excitability, as measured by a third shock in the same direction as the first, should show hardly any change from the resting condition, provided that the initial shock was less than $\sqrt{h}$ threshold intensity. If this value is exceeded, however, the resulting excitability curve should be that due to the local action potential alone. If the third shock is sent in the same direction as the second it will test the action potential (if any) which arises from the anode.

b—Constant Currents

Fig. 6 shows the curves obtained by the graphical analysis of constant currents whose intensity in multiples of the rheobase is indicated by the number on each curve. It is seen that subrheobasic currents cause an exponential rise of θ to a final level proportional to the current but never rising higher than the transitional value $h\theta_1$ for short interpolar length, or $\sqrt{h}\,\theta_1$ for great interpolar length. Currents greater than the rheobase give rise to curves which, below the transitional value, are the same as the foregoing except that the ordinates are proportionally greater, but above this value the curve changes from concave to convex.

To establish the exact form of this upper portion consider the following operation. Suppose that we perform the graphical analysis of the first part of the curve again, but this time on a piece of tracing paper, and with every thing reduced in scale in the ratio $(1-h)/h$ (for the case of short interpolar lengths). The curve obtained will naturally be the same as before, but similarly reduced. The analyser used will have subtangent $\frac{\alpha}{h}(1-h)$ and hence will be the analyser used for the second portion of the curves in fig. 6. Now, suppose that we take the tracing paper and put it upside down on fig. 6, with the horizontal axis coincident with the upper horizontal of fig. 6, then it is easy to see that the two dotted horizontals must coincide, and the line I_1/I_0 on the tracing paper will coincide with the "reflected curve".

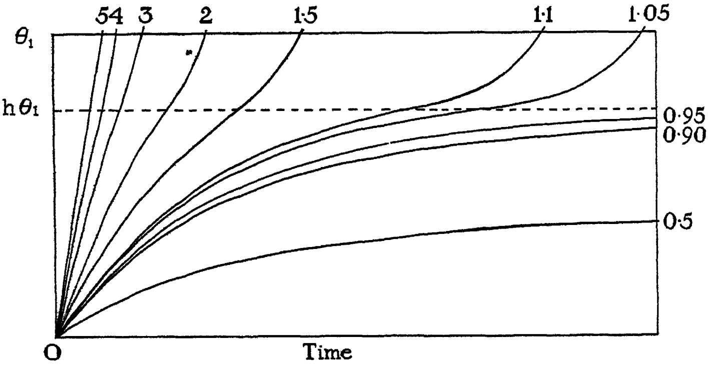

FIG. 6—The time course of θ following the making of a constant current at time O. For comparison with the experimental results of Rushton (1932) which are plotted the other way up.

It follows that the second part of the curve in fig. 6 must be the same as the curve on the tracing paper, since they are both derived by analysing the same curve by the same analyser between the same limits. And thus the portion of the curve above the dotted horizontal is similar to the portion below, and similarly situated with respect to the point of intersection with this horizontal; they stand in the proportion of the two subtangents.

The same result holds for great interpolar lengths, allowing for the approximation made in obtaining equation (16).

It follows from the geometrical symmetry of the two parts of the curve, that the time taken for θ to attain θ_1 is a constant multiple of the time taken to attain the transitional value, this constant being

$$1/h \quad \text{or} \quad 2/(1+h) \text{ for short or great lengths.}$$

Consequently the strength duration curve has the form

$$\frac{I_0}{I} = 1 - e^{-\frac{ht}{\alpha}} \quad \text{or} \quad \frac{I_0}{I} = 1 - e^{-\frac{1+h}{2\alpha}t}, \tag{19}$$

where I is the current and I_0 the rheobase.

This is the condenser formula of excitation which has been proposed countless times. Evidence is rather conflicting as to its accuracy, but on the whole it is probably a good approximation to the experimental data. Where the present theory differs from the classical condenser concept is that the time constant of the physical process α is significantly smaller than that of the excitation process.

A more striking difference is seen in the curves of the way that θ develops under the action of a constant current. According to the condenser theory all curves should run as do those in fig. 6, below the transitional value, and excitation should occur when this value is attained. Exactly this has been described by Bishop (1928), who was the first to investigate the matter by the method of superposed shocks. Unfortunately he restricted himself to subrheobasic currents, which do not exhibit the characteristic inflexion, and differ from the condenser theory expectations only in that a just subrheobasic current requires a moderate shock to be superposed, and not an infinitesimal one. This Bishop found, but explained it away by an alleged property of his circuit which appears to be inconsistent with Kirchoff's laws. Erlanger and Blair (1931*b*) repeated the observation and disproved, for their circuit, Bishop's explanation. They were chiefly interested in questions of accommodation to a polarizing current and also confined themselves to subrheobasic currents. Their curves which plot, for the most part, action-potential heights, are not strictly comparable with the curves of the present analysis, but in a general way they agree over short durations where accommodation is not significant.

At the same time, but unaware at first of their investigations, I made a number of similar observations, much cruder in method, but extending into the range of super-rheobasic currents. The curves obtained showed the inflexion as in fig. 6, and were obviously inexplicable on the simple classical theories. I was unwilling to publish these without being able further to explain their significance, and merely mentioned the matter in a short note (Rushton 1932) wherein the curves are seen to be extremely similar to those of fig. 6 plotted the reverse way up. More detailed discussion of these and related results will be presented in a separate paper.

c—Condenser Discharges

The condition that a condenser discharge should be just threshold is easiest established from the geometry of fig. 7, where A and B are two exponential curves asymptotic to RS whose ordinates RP and RQ are in a constant ratio. OPS is the tangent at P, hence from the property of the exponential RS the subtangent is constant. Thus from similar triangles OQ is also constant and independent of the position of P.

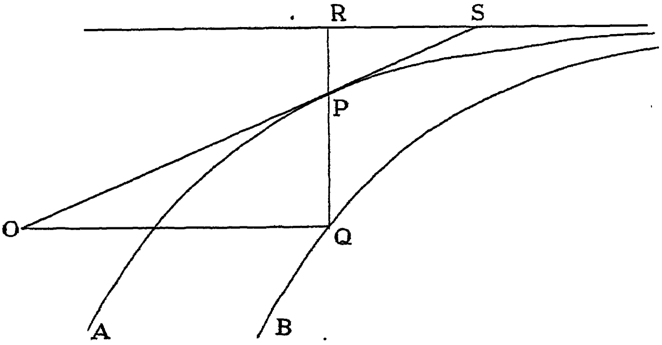

FIG. 7

Now the "reflected curve" in the graphical analysis of a condenser discharge is an exponential of the form B, and hence it is clear from the construction, that if the curve of θ in the second range of analysis starts by lying on A, so situated with regard to B that OQ = subtangent of the second analyser, it will continue to trace out A indefinitely. Obviously this is the threshold condition since the slightest deviation on either side of B will cause the curve of θ quickly to diverge much farther.

We thus conclude that the utilization period of a threshold condenser discharge is the full period of the discharge. This is not experimentally the case. There is uncertainty as to the exact experimental relation but agreement that an appreciable fraction of a slow discharge may be cut off without affecting the efficacy. Three factors at least may enter into this divergence from the theory. Conditions are nearly always slightly super-threshold and in this case the utilization period is very considerably diminished. Measurements are not usually made with electrodes very close together, and so the theoretical results are invalidated by the approximation introduced into equation (16). Accommodation will diminish the utilization period. According to the recent results of Fabre (1933) a condenser continues to act until its voltage has fallen well below rheobasic voltage, but not to zero, it therefore fits the present theory as modified by accommodation, etc. It will not, however, fit the classical condenser theory, according to which a subrheobasic intensity can never be present at the instant preceding excitation.

The voltage-capacity relation for threshold condenser discharges has been investigated by countless workers; the recent experiments of Hill (1936*b*) are probably the most accurate. He found that the relation fitted precisely the theoretical curve obtained on the classical condenser theory, hence it is important to find what curve is to be expected upon the present theory. Unfortunately the working, though straightforward, is rather long, and I shall therefore merely mention the method and quote the result.

Taking the case of large interpolar distance, as in Hill's experiments, we introduce into (14) V_0 for I_0 and $Ve^{-t/\beta}$ for I_1, where β is the time constant of the condenser discharge. Putting X_2 zero we obtain the value t_0 when $\theta = \theta_1\sqrt{h}$. Now introducing the threshold conditions of fig. 7, we may eliminate t_0 and obtain

$$\log\left(\frac{V}{V_0}\right) = \frac{1}{1-\dfrac{\beta}{\alpha}}\left[\log\left(\frac{2\dfrac{\alpha}{\beta}}{1+h}\right) - \frac{\beta}{\alpha}\log\left(1+\frac{\alpha}{\beta}\cdot\frac{1-h}{1+h}\right)\right]. \qquad (20)$$

When $h = 1$ this reduces to Hill's formula.

Table I shows the result of computing the value of $\log_{10}\left(\frac{V}{V_0}\right)$ for various values of $\log_{10}\left(\frac{\beta}{\alpha}\right)$ where $h = 0{\cdot}75$. The third line gives the value of $\log_{10}\left(\frac{V}{V_0}\right)$ according to Hill's formula, where V_0 is the same in the two cases, but in the second α has been replaced by $2\alpha/(1+h)$. It is seen that the two curves never differ by more than 0·026 and thus the results of Hill's experiments fit the present theory as accurately as they do the simpler formula which

TABLE I

$\text{Log}_{10}(\beta/\alpha)$	2	1	0	−1	−2	−3
$\text{Log}_{10}(V/V_0)$ (present theory)	0·02	0·11	0·44	1·134	2·07	3·06
$\text{Log}_{10}(V/V_0)$ (Hill's theory)	0·02	0·12	0·46	1·160	2·07	3·06

he applied. Fabre's results, on the other hand, certainly favour the present theory.

The time constant $2\alpha/(1+h)$ of the voltage-capacity curve (Hill's k) is interpreted differently on the two theories. According to the condenser theory this is the constant of the charging process in the nerve, but on the present theory α is the charging constant. The same difference was found above (19) in the case of constant current stimulation. According to the present theory, therefore, a nerve should have the same form of curve both for physical charging and for excitation, but the latter should have a somewhat longer time constant. Exactly this result has been found by Harris and Rosenberg (1935) if the development of a given electrotonic potential be regarded as a measure of the physical charging. Since, however, the assumptions of the present theory require that the charging of the membrane should affect infinitesimally the electrotonic potential, it is perhaps unjustifiable to claim support from Harris and Rosenberg's evidence. However, Katz (1937) has found that the charging rate, as measured by the exponential decay in the excitation remainder following a single weak shock, corresponds to the rate found by Harris and Rosenberg and is considerably smaller than the constant derived from voltage-capacity excitation curves.

d—Evaluation of Constants

We are now in a position to assign numerical values to the constants of the foregoing equations. From equation (9) we have

$$v = \frac{hL}{\alpha(1-h)},$$

where v = velocity of conduction = 30 mm./msec. at 20° C.

L = length constant for nerve in air = 3 mm. (Rushton 1934).

$\frac{2\alpha}{1+h}$ = Hill's k = 0·34 msec. at 20° C. (Hill 1936*b*).

Substituting the above values we obtain

$$h = 0{\cdot}75, \quad \alpha = 0{\cdot}3 \text{ msec.}$$

This is the value which has been assumed for h above in making approximations.

The Safety Factor (10) $= \frac{h}{1-h} = 3$.

The Liminal Length (8) $= -L\log_e h = 0{\cdot}86$ mm.

As it happens this is about the distance between one Node of Ranvier and the next.

2—SPATIAL CONSIDERATIONS

If the current is led to and from a nerve by conductors which are not small compared to the nerve trunk, we may assume that the current is distributed around each fibre with radial symmetry. The current then passing across the sheath is given by equation (2) where E is put zero. This formula is essentially the same as the one I put forward (1928) for nerve in fluid, and showed to satisfy certain variations in the spatial distribution of stimulus. The constant L, deduced in the first place from excitability measurement, should have the physical significance $\sqrt{\{R/(r_1+r_2)\}}$. This comparison has been made (Rushton 1934), and, though the accuracy of the experiments was not very great, there was no detectable difference between L as found by resistance and by excitability measurement. The present theory, therefore, appears to account for the general spatial as well as for the general temporal aspects of excitation. Three qualifications, however, must be made.

First, the value of L is assumed to be the ratio of purely ohmic resistances. But we know that nerve has capacity, and R is presumably less for brief than for longer shocks. I have in fact found that L is less with briefer stimuli, and several workers (Cardot and Laugier 1914; Bouckaert and Katz 1935, discussed by Hill (1936*b*)) have found that the time constant of the strength-duration curve is less with short than with long interpolar lengths, which is another way of expressing the same thing. As we have seen (19), even excluding capacity, some difference in time constant is to be expected with great or small interpolar lengths, from the different equations applicable in these two cases. But the effect thus deduced is small, and the sense opposite to that observed.

The second qualification is due to the connective tissue sheath which deflects the current in a manner distinctly appreciable at short inter-electrode distances.

The third refers to tripolar electrodes. As has been shown in (18), in the symmetrical case, if the distance between adjacent electrodes is less than

$$L\log_e\left(\frac{1+h}{2h}\right) = 0{\cdot}5 \text{ mm. (approx.)},$$

propagation cannot take place. This result may be related to the well-known observation of Pratt (1930) and Gelfan (1930) that a micro-electrode intimately applied to a single muscle fibre gives rise to a localized contraction which will not propagate. Conditions increasing the length excited allow of propagation. Now the current leaving the fibre at the microelectrode must enter it elsewhere and in fact it enters through the regions just either side of the electrode, thus forming a system not unlike the tripolar arrangement from which (18) was deduced.

Two other characteristics of the localized contraction in the basihyoid membrane, mentioned by Kato (1934), support the present interpretation. First, whenever the localized contraction spread more than 5 mm., it propagated throughout the whole fibre. Second, the localized effect was less prominent in fresh muscles than in those kept some time in Ringer's fluid, or especially those narcotized. Thus, conditions lowering the Safety Factor, as we shall deduce below in (21), increase the liminal length, and the phenomena localized within it.

According to the assumption of an action potential persisting indefinitely, it is obviously impossible for the impulse to be propagated in one direction only from the point of stimulation, for, as the active length becomes great enough, the region on the other side of the electrode will propagate. This does not follow, however, if we take into account the brief duration of the action potential. If the cathode is far from another electrode, conditions will be symmetrical on either side, but if the anode is near, the initial propagation (as we have seen) will be entirely into the extrapolar region. At the moment when the stimulus is removed, propagation will continue into the extrapolar region which is still cathodally charged, but hardly in the other direction where the charge is still anodal. Thus, in a short while, when the first stretch has become inactive, the new active stretch in the extrapolar region will be considerably longer than the new stretch in the interpolar region. And if the former was barely sufficient to continue propagation (which is the threshold condition) the latter will be insufficient, and propagation will occur in the extrapolar direction only. This will only happen with short interpolar distance and threshold strength (unless we consider very strong currents with the classical anodal block).

3—ELECTRIC RESPONSE

Any satisfactory theory of nerve activity should describe and if possible explain the form of the action-potential wave. In the present exposition this whole matter has been sacrificed for the sake of a relatively simple treatment. If the theory turns out to be of value, this is the direction in which it will most need development.

One further matter in connexion with the electric response, however, needs consideration. Should an active stretch just less than the liminal length give rise to an appreciable action potential? Many workers have looked for and failed to find an action potential resulting from a just subthreshold stimulus. Blair and Erlanger (1936*a*), considering just such a possibility as is worked out in this paper, conclude that the action potential associated with an active length less than liminal cannot exceed 2 % of the full spike.

To find the value to be expected on the present theory we note that, in the absence of external stimulus, the stimulation at X_2 due to the active region from $-X_1$ to X_2 is

$$K_1(1-e^{-\frac{X_2+X_1}{L}}),$$

where K_1 is the stimulation when X_2+X_1 is great, i.e. the stimulation due to a full-grown impulse. But if F is the Safety Factor, then by definition $K_1/(F+1)$ is a stimulation which will barely propagate, and is thus equal to the stimulation produced by the liminal length X.

Thus
$$K_1(1-e^{-\frac{X}{L}}) = \frac{K_1}{F+1},$$

$$e^{\frac{X}{L}} = 1+\frac{1}{F}. \tag{21}$$

But the action potential from the mid-point of an active region of length X is

$$K_2(1-e^{-\frac{X}{2L}}),$$

where K_2 is the potential from an active region extending far in each direction. Hence, when X is the liminal length, the ratio of the "liminal action potential" to the maximum action potential is

$$1-e^{-\frac{X}{2L}} = 1-\left(1+\frac{1}{F}\right)^{-\frac{1}{2}}. \tag{22}$$

Now in the section "Evaluation of Constants" the safety factor came to 3, which when substituted in (22) gives the liminal action potential as 13·5 % maximal—a value far too great to have escaped observation by Blair and Erlanger. On the other hand, the safety factor is certainly greater than 3. Conduction early in the refractory period and in anodal block suggest that 10 is a better approximation (Hodgkin 1937) and substituting this in (22) gives 4·7 % maximal for the liminal action potential. Moreover, it can easily be shown that if this value is approximately true and the stimulus is *n* % below threshold, then the resulting action potential expressed in terms of maximal will also be n % less than in the threshold case. Thus a 97 % threshold shock in the case above would cause an action potential only 1·7 % maximal, and be within Blair and Erlanger's limits of error.

Since the excitation of the liminal length appears to give rise to an action potential too small to be recorded, and since the velocity of propagation in these circumstances is practically zero, a finite latent time will elapse before the action potential reaches a visible size. This latency has been found by Blair and Erlanger (1936*a*), but the capacity of the tissue must also play a large part, as they suggest.

The present theory will never stand upon a firm basis until a subthreshold shock has been shown to leave a localized action potential, and to be followed by a refractory period. The treatment in this paper is too simplified for quantitative estimates to carry much weight, but it does appear possible that the liminal action potential may lie below the limits so far recorded, but that a small extension of those limits will reveal it.

4—ACCOMMODATION

Hill (1936*a*) and Solandt (1936) have shown that the relative inefficacy of currents of long duration is accurately accounted for by assuming that the threshold for excitation is not constant, but rises according to a first order differential equation. It is possible to interpret this by supposing that there is some polarizable structure in series with the excitable element, and that the observed inefficacy of slow currents is due to their partial neutralization by the polarization currents.

This concept may be applied equally to the present theory. I have recently shown (Rushton 1937) how to use a graphical analysis to solve Hill's equation for the effect of accommodation, and this same method may be applied in the two ranges of graphical analysis in the present paper, assuming that a nerve accommodates to its own action potential in the same way as to an applied stimulus. It is obvious that in this way accommodation may be brought within the scope of the present theory. This has not been included in the Mathematical Section, however, partly for simplicity, but partly because there is an altogether different phenomenon which must also be considered.

If the action potential does not continue indefinitely, but only for some 2 msec., an adequate stimulus must not only be able to raise to activity the liminal length, but it must do this rapidly, otherwise the region first excited will already have become refractory before propagation has reached the liminal length. Now the classical idea of accommodation was that unless the critical polarization was accomplished within a brief time, the strength of stimulus had to be greatly increased, so we may ask whether accommodation can be explained entirely on the basis of the refractory period of the first excited portion of tissue.

That refractoriness and accommodation are connected is strongly suggested by the comparisons of Erlanger and Blair (1931*a*) between the relative refractory period and the post-cathodal depression following a single subthreshold shock. But refractoriness can hardly explain the anodal excitation at the opening of a constant current, and refractoriness is, I suppose, still present in a citrated nerve where accommodation is practically absent.

It therefore appears that two distinct phenomena may enter into accommodation, first, the refractoriness of the portion of nerve first excited, and second, the polarization of some structure by both the stimulus and the subthreshold action currents. The relation between relative refractoriness and post-cathodal depression may thus have a twofold basis, for the latter may be partly caused by the *localized* refractory period, while the *propagated* refractory period may be due in part to polarization from the action current. We should thus expect the post-anodal enhancement following a single just subthreshold shock to differ in shape from the post-cathodal depression, assuming the former to be uncomplicated by a localized activity followed by a refractory period. The effect at the cathode should therefore show a greater and more prolonged enhanced period followed by a more marked depressed period. This difference is seen in fig. 7 of Erlanger and Blair's paper.

DISCUSSION

The present theory has, for simplicity, made two very serious errors in assumption. First, the nerve is taken as non-capacitative, and thus all the temporal aspects of electrotonus cannot be considered. Second, the action potential is assumed to rise instantly to its full value and persist indefinitely, consequently considerations relating to the form of the observed wave cannot apply. In addition these two erroneous assumptions affect to some extent the quantitative validity of all the conclusions of this paper, as has been pointed out in many places above. The results must also be modified by considerations of accommodation and localized refractory period (as just discussed).

Finally, it must be stated that there are other and quite different sets of assumptions which will give, in a general way, most of the results of this paper. The value of the present assumptions is that they are necessary deductions from the generally accepted theories of excitation and propagation.

In spite, however, of its errors and limitations, the theory has something to its credit. It yields, in a fairly manageable form, the results of stimulation with currents of any temporal form whatever, applied in any spatial distribution whatever. It describes in a quantitative and verifiable way the process both of excitation and of conduction. If we are given the velocity of conduction and length constant L from physical measurements, and also in given circumstances the rheobase I_0 and time constant (Hill's k), we have all the constants required for the entire working of the theory. Of these four constants only I_0 and k are arbitrary, the other two are physical measurements, thus, in spite of its scope the theory has no more arbitrariness than was formerly required to describe the strength-duration curve alone. One may even hope that, with the introduction of assumptions as to capacity into the present theory, the constant k may be deducible from physical measurements upon charging rate.

With regard to excitability relations, the theory will describe the strength-duration and voltage-capacity curves, with an accuracy as great as that of the experiments, and will account for the results of superposing shocks in a way that invites attention.

The chief claim of any theory, however, lies in its suggestions for further investigation. To this end the present ideas have been put forward with as definite a physical significance as possible. In this way the concepts become sharper, more provocative to those inclined to attempt disproof, more clearly verifiable for those attracted. The theory offers suggestions for experimental correlation over a wide field, and if this is carried out, our knowledge should be appreciably extended.

The ideas developed in this paper have arisen largely out of informal discussions with Mr. A. L. Hodgkin, for whose criticisms and suggestions it is a pleasure to express my indebtedness.

SUMMARY

1—We assume that a certain depolarization of a nerve at a point is the necessary and sufficient condition that an element of action potential be generated at that point, and that propagation results from the stimulation of the inactive region in front of the propagated wave by the spread of the action current. It follows that excitation is inadequate unless it

activates a sufficient length of nerve to give an action current big enough to propagate.

2—In Part I of this paper the matter is worked out on an over-simplified scheme. The "Mathematical Section" is separated from the "Assumptions" and the "Conclusions from the Mathematics", so that those not interested in the technical treatment may yet see in summary form both what is assumed (p. 212) and what is concluded (p. 227).

3—In Part II the theory is applied to a selection of the known observations upon excitability. The strength-duration curve, the voltage-capacity curve, and the effect of two successive shocks, are satisfactorily described, including certain aspects which will not fit the classical theories. The spatial relations of excitation are also satisfactorily described, including an explanation of the non-propagated contraction arising from microstimulation of single muscle fibres.

4—The theory, though too simplified in the present form, suggests a large number of experiments, in the light of which further development should be possible.

REFERENCES

Bishop, G. H. 1928 *Amer. J. Physiol.* **84**, 417.
Blair, E. A. and Erlanger, J. 1936*a* *Amer. J. Physiol.* **114**, 309.
— — 1936*b* *Amer. J. Physiol.* **117**, 355.
Bouckaert, J. P. and Katz, B. 1935 *J. Physiol.* **85**, 10*P*.
Cardot, H. and Laugier, H. 1914 *C.R. Soc. Biol., Paris*, **77**, 276.
Erlanger, J. and Blair, E. A. 1931*a* *Amer. J. Physiol.* **99**, 108.
— — 1931*b* *Amer. J. Physiol.* **99**, 129.
— — 1933 *Amer. J. Physiol.* **106**, 565.
Fabre, P. 1933 *C.R. Soc. Biol., Paris*, **112**, 1425.
Gelfan, S. 1930 *Amer. J. Physiol.* **93**, 1.
Harris, D. T. and Rosenberg, H. 1935 *J. Physiol.* **84**, 11*P*.
Hill, A. V. 1936*a* *Proc. Roy. Soc.* B, **119**, 305.
— 1936*b* *Proc. Roy. Soc.* B, **119**, 440.
Hodgkin, A. L. 1937 *J. Physiol.* (in the Press).
Kato, G. 1934 "Microphysiology of Nerve." Tokyo.
Katz, B. 1937 *J. Physiol.* **90**, 6*P*.
Pratt, F. H. 1930 *Amer. J. Physiol.* **93**, 680.
Rushton, W. A. H. 1928 *J. Physiol.* **65**, 173.
— 1932 *J. Physiol.* **75**, 16*P*.
— 1934 *J. Physiol.* **82**, 332.
— 1937 *Proc. Roy. Soc.* B, **123**, 382.
Solandt, D. Y. 1936 *Proc. Roy. Soc.* B, **119**, 355.

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Experimental Evidence for a Non-conducted Response of Nerve to Subthreshold Stimulation

By Bernhard Katz

Physiology Department, University College, London

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Introduction

It is assumed in most theories of excitation (e.g. Hill 1936*a*) that, when a critical state is reached at a *point* of the nerve, "excitation" occurs and then automatically propagates over the whole length of the nerve fibre. Since, however, the self-conduction of an impulse involves stimulation of each element by the simultaneous activity of a finite adjacent region of nerve, it is more natural to suppose that initially also a certain *minimal length* of nerve must be excited by an applied shock, in order to give rise to a propagated disturbance. A subthreshold stimulus, therefore, by exciting too small a region, might produce a localized response, the spread and size of which are not enough to excite resting points further on.

If such a non-conducted response can actually occur, it might be expected (i) to facilitate excitation by a second shock, if applied to the same region shortly after the first, and (ii) to be accompanied by a small local action potential. Both possibilities can be investigated experimentally, though both might be expected to be complicated by the direct effects (electrotonus, "local potential") of the first shock.

The suggestion of a local response may be obvious intuitively; it follows, however, directly from the theory of electric transmission of the nerve impulse, as put forward, for example, by Hermann, Cremer, Rashevsky, and Hodgkin, and it has recently been worked out in detail by Rushton (1937*c*).

The problem was first approached experimentally by determining the time course of the local excitatory process in nerve, using the "double-shock" method. Later, direct electric evidence was sought of the local unpropagated response.

I—THE LOCAL EXCITATORY DISTURBANCE FOLLOWING A BRIEF SHOCK

Excitability measurements by the successive application of two shocks have frequently been made (e.g. Gildemeister 1908 and Erlanger and Blair 1931): usually the effect of a subliminal "conditioning" shock was tested by applying, after various intervals, a second superthreshold shock and measuring the changes produced in the size of response (muscle twitch—Gildemeister: action potential—Erlanger and Blair). This method yields good qualitative information, and reveals, moreover, the moment at which a period of enhanced or depressed excitability is terminated or reversed. During the present investigation, however, the size of response was kept constant and the change of threshold was measured.

Method

Electric Circuit—Repetitive brief condenser discharges were used as conditioning and as testing stimuli (S_1 and S_2 respectively). The circuit is shown in fig. 1. The time constants RC of the shocks were of the following order: At room temperature, S_1, 3–10 μsec.; S_2, 13–20 μsec.; at low temperature, S_1, 5–20 μsec.; S_2, 24–40 μsec. The testing shock S_2 was necessarily slower, because, with the arrangement used, its discharging capacity was determined by (C_1+C_2). The stimuli, in particular S_1, were effectively within, or very close to, the range in which the "constant quantity relation" holds. The strength, therefore, of the conditioning shock S_1 could be adjusted by means of a variable calibrated condenser C_1, while keeping the voltage V_1 at a fixed value. When working with conditioning shocks only one or a few per cent above or below threshold, it proved very convenient to adjust the required strength by a Sullivan air condenser, the scale of which gives divisions of 5 $\mu\mu$F. The values of the voltages, capacities and resistances employed are given in the legend of fig. 1.

*Measurement of Interval**—For many experiments, a Pye commutator with two adjustable rings on the same shaft was used, at about 20 rev./sec. The interval between the two shocks was varied by shifting the middle brush around the surface of one ring, and measured after each setting with an electric method as described by Bugnard and Hill (1935). Intervals could be employed down to about 70 μsec., though they generally ranged between 100 and 2000 μsec.

The accuracy of the method was limited by a certain "zero error" involved in determining the interval. If the contacts were watched carefully and kept clean, this error was small and usually well below 30 μsec. Actually, the difference between the time constants of conditioning and testing discharge introduced an apparent "zero error" of opposite sign, so that the net error was small compared with the time taken by the changes observed, provided that the experiment was made at a low temperature.

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* (*Added in proof.*) Owing to an error in the calibration, the absolute time values given below require a correction of +9%.

In a later series of experiments, a commutator, designed and constructed by Mr. A. C. Downing, was used. It contains several rings fixed on the same shaft. The interval could be adjusted, without stopping the motor, by sliding the contact brushes around the surface of the running commutator rings, and was read directly by means of a pointer sliding, together with the brushes, along a concentric millimetre-scale, of 12 in. radius. At 20 rev./sec., therefore, each division corresponded to about 25 μsec. The scale was calibrated, using a microammeter, as in the other experiments; in the same way, the "zero position" of the brushes was frequently tested.

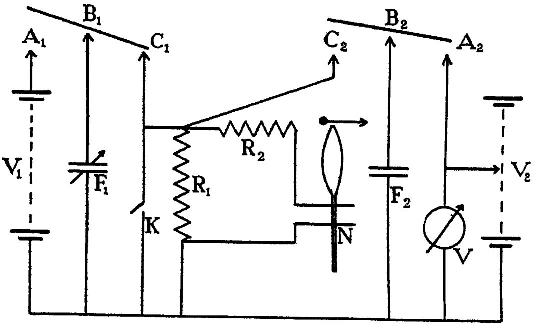

Fig. 1—Diagram of circuit used for the application of two short shocks. A_1, B_1, C_1 and A_2, B_2, C_2, terminals of the two respective commutator rings. V_1, V_2 and F_1, F_2, respective voltages and capacities (F_1 and V_2 variable; voltages, about 10–20 V; condensers, 3–10 mμF.). R_1, 1000–2000ω shunt; R_2, 50000ω series resistance. V, voltmeter. N, nerve with muscle and attached lever. K, Morse key; stimulation when K is opened.

Preparation—Sciatic-gastrocnemius preparations of Hungarian *Rana esculenta* were used, and minimal muscle response was taken as threshold index. Calomel electrodes and paraffin-wax chamber, as described by Hill, Katz and Solandt (1936), were employed.

Results

The principal results will be illustrated as in fig. 2, threshold difference between "resting" and "conditioned" nerve being plotted against interval between conditioning and testing shock. In this way, the time course of the "local excitatory disturbance", as it is usually called (Lucas 1910;

Hill 1936 *a*, *b*), is represented, its amount, at any moment, being proportional to the ordinate. Recent excitation theories (Hill, Rushton), however, attribute a more explicit meaning to such "excitability" curves, which must be considered before going into experimental details.

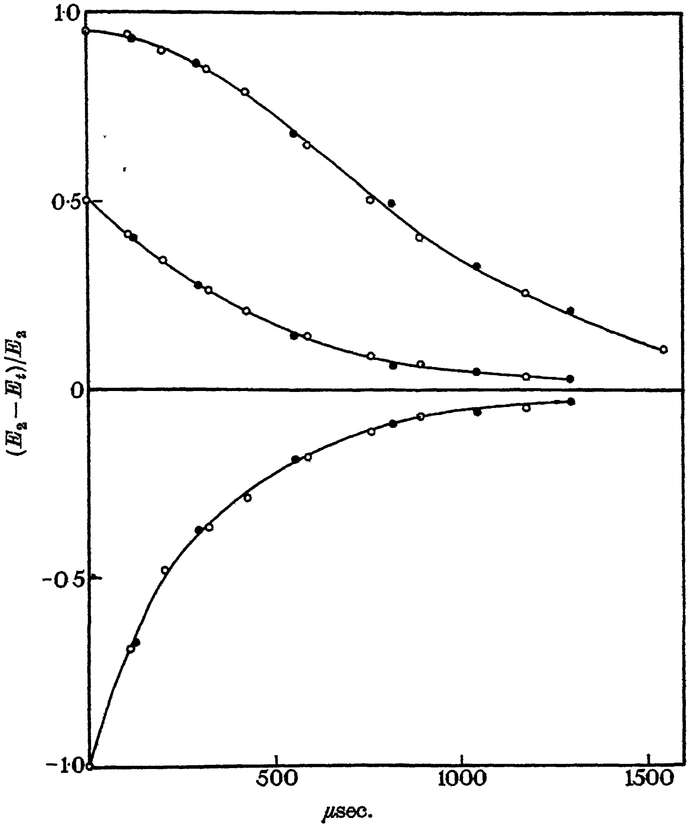

FIG. 2—"Excitability curves" obtained with three different conditioning shocks E_1. Strength of E_1, successively from above: 0·95, 0·5; −1·0. Experiment of 30 April 1937. Temp. 5·5° C. In this figure and in figs. 3, 4, 9, hollow circles: series of observations from short to long intervals; full circles: reverse series. For further explanations see text.

According to Hill (1936 *a*, *b*), a very short shock raises the "local potential" under the cathode above its resting level V_0 by an amount proportional to the applied electric charge, and then V falls exponentially to its initial value, with a time constant characteristic of the stimulated nerve. If V ever reaches a critical level U, then excitation occurs. The ordinates of fig. 2 should give us, therefore, at any moment, neglecting a slight accommodative rise of U, a relative measure of the alteration of "local potential' (V–V_0) left by the conditioning shock; the curves should all be similar and should represent the exponential decay of (V–V_0) immediately under the cathode. Evidently this may apply quite well to the two lower curves in fig. 2, but not to the upper one obtained after a shock 5 % below threshold, which has a definite "hump".

The situation becomes somewhat different if we assume that a short shock must excite a certain minimal length of nerve in order to produce a propagated response (Rushton 1937*c*). The strength of the test shock must then, at any moment, be adjusted so as to produce a critical depolarization (in Hill's terms: to make V equal to U) at the margin of this critical length. If the excited region were too small, then a non-conducted response might occur, which, though unable to propagate itself, might sum at any moment with the remaining "local potential" and lower the threshold for a second stimulus. "Excitability" curves as in fig. 2 would then trace the time course of the "total excitatory disturbance" at some critical distance from the cathode (i.e. the sum of (i) an exponentially decaying "local potential" and (ii) an assumed "local response" (or "local action potential") generated by the activity of a subliminal length of nerve).

On this hypothesis the meaning of fig. 2 becomes evident: the two lower curves show the simple exponential decay of the "local potential", while the "hump" of the upper curve (conditioning shock 5 % below threshold) represents the "local response".

Actually the spread of "local potential" produced by an instantaneous shock, over the critical distance considered, would not take place instantaneously. There would be a certain lag between the end of either shock and the attainment of $V_{\max}$ at this distance. Judging, however, from Bogue and Rosenberg's (1934) results the initial delay in the development of electrotonus at 1 or 2 mm. distance from the cathode occupies only 0·1 msec. or less, and would be the same for test shock as for conditioning shock.

The results, according to strength and sign of the conditioning shock E_1 ($E_1 = 1{\cdot}0$ being threshold), can be divided into four groups:

(i) weak cathodic shocks, producing merely a "local potential", but no detectable response;

(ii) stronger but still subthreshold shocks, after which a local response can be traced;

(iii) anodic stimuli, which, of any strength, merely build up a—negative—"local potential"; and

(iv) superthreshold shocks, which elicit self-propagating responses.

The majority of the experiments were made at low temperature, at which, owing to the larger time scale of the nerve, more accurate observations were obtained. Apart, however, from the absolute value of the time factor, no essential differences were found between different temperatures.

Group (*i*)—Using cathodic conditioning shocks of half or less than half-threshold strength, the fall of the "excitability curve" follows, within the limits of experimental error, a simple exponential relation (figs. 2–4). The time factor was determined from the slope of the straight line which results from plotting semilogarithmically. Comparing the effect of $E_1 = 0{\cdot}5$ and $E_1 = 0{\cdot}3$ in the same nerve, similar exponential curves, with identical time constant, are found. This satisfies the fundamental equation of Hill's "local potential" theory (Hill 1936*a*, p. 317): $V-V_0 = bQe^{-t/k}$. The time constant k, however, is smaller than the ordinary values obtained from a voltage-capacity curve when taking a propagated response as threshold index. The mean value of fifteen experiments at 19·5° C. was 180 μsec. (varying between 110 and 345 μsec.); at 5° C., thirty-seven experiments had a mean k of 400 μsec. (210–635 μsec.). These figures are only about one-half of the ordinary k values; they agree, however, more closely with the time constant found by Harris and Rosenburg (1935) when taking a catelectrotonic peak potential as index, and not a propagated response. At room temperature, Harris and Rosenberg found $k = 170$ μsec.: at low temperature, comparable data are not available yet.

Remembering, however, that "excitability" tests presumably refer to events at some little distance away from the cathode, the time constants of electrotonus, and of "local potential" as determined in these experiments, may not be strictly comparable.

It is of interest to compare the temperature coefficients of these three phenomena, especially since it has been pointed out (Monnier 1934; Gasser 1937) that the electrotonic potentials are much less affected by temperature changes than any "true" excitatory process (Bogue and Rosenberg found for the rate of rise of the electrotonic potential a Q_{10} of 1·2, Hill (1936 *b*) a Q_{10} of 1·8 for his time factor k). The time factor of the "excitability decay" showed—rather like that of ordinary strength-duration curves—considerable, individual and seasonal, variation: "winter frogs" at 18° C., average $k = 185$ μsec., at 4·5° C., average $k = 480$ μsec.; "spring frogs", at 21° C., average $k = 175$ μsec., at 6° C., average $k = 280$ μsec. Obviously, the influence of temperature must be studied on the same nerve. This was facilitated by the use of Downing's commutator, allowing a much quicker performance of each experiment. A preparation was soaked for a few hours, and then either (*a*) was set up successively on electrodes in separate cold or warm chambers, or (*b*) was left in a single chamber used successively at two temperatures. The first procedure was quicker, because it avoided the slow cooling of the chamber; the second, however, was more reliable, since possible local variations of k along a nerve were eliminated.

A summary of these experiments is given in Table I. The temperature coefficient varied, even in the experiments where no progressive change was found; the average Q_{10} in the last four experiments (the most reliable) was 1·5, definitely smaller than that of k obtained from the strength-duration curve (Hill 1936*b*), though larger than Bogue and Rosenberg's value.

TABLE I—TEMPERATURE COEFFICIENT OF DECAY OF "LOCAL POTENTIAL"

Experiments with two separate, hot and cold, chambers

Temp. ° C.	22	6·9	21·5	6·9	21	6·3	19·8	6·4	20	5·5	21·5	6·3
k, μsec.	140	240	110	265	195	260	345	285	125	440	240	205
Q_{10}		1·55			1·23							

Two preparations of same frog. Gross progressive change (shortening of k) prevents Q_{10} determination

Experiments with single stimulating chamber

Temp. ° C.	5·2	22·4	5·0	5·8*	7·3†	21·2	4·5	3·8	20	4·5	7	21·3	6
k, μsec.	325	145	295	250	245	120	315	300	170	275	260	155	270
Q_{10}		1·55			1·64			1·4				1·44	

* 2 hr. interval. † 2½ hr. interval.

The fact that this time constant, as well as the electrotonic one, is smaller than Hill's k, is readily explained by the intervention of a local response (cf. Rushton 1937*b*, *c*). A similar explanation has been proposed already by Harris, Rosenberg and Sager (1936). A rheobasic current, even if it excites primarily only a minute region, would foster the new-born local response and help it to spread over adjacent parts, till it reaches propagating strength. A short shock, however, unless it be made so strong that it immediately excites the whole critical length, would generate a local response, which, finding little support by the rapidly decaying "local potential", would be too feeble to propagate. The amount of "local potential", therefore, required for *propagation*, is higher with short shocks than with constant current; and, since the ratio Q_0/I_0 (minimum electric charge with short shocks/minimum constant current) determines the time factor k of the strength-duration curve (Hill 1936*a*), k will be found larger, when the required index is a propagated response, than it is for a given "local potential".

The difference in Q_{10} might also be explained on this basis: According to Gasser (1931), the strength of the impulse decreases with temperature (below 20° C.). If the activity at each point of a nerve is weakened, then clearly a brief shock must excite a greater length of nerve, if it is to produce a sufficiently strong, self-supporting response. Consequently, the discrepancy between the two time factors should be greater at low temperature, which would lead to the different values of Q_{10}.

Group (ii)—When the strength of the conditioning shock E_1 is greater than 0·5, the form of the curve changes (figs. 2–4). There are conspicuous "humps" with $E_1 = 0{\cdot}85$ and above; with intermediate intensities ($E_1 = 0{\cdot}5$–$0{\cdot}75$) one may find merely a slowing of the excitability decay, without very obvious change in its exponential form; a just perceptible delay, however, is often present even with $E_1 = 0{\cdot}5$. These deviations occurring only at the cathode, and detectable only above a certain strength of stimulus, indicate the presence of the predicted local response, adding its effect to the exponentially decaying "local potential". To trace the shape of the former, all that is necessary is to subtract the latter, the time constant of which was found with weak conditioning stimuli, from the "total excitatory disturbance".

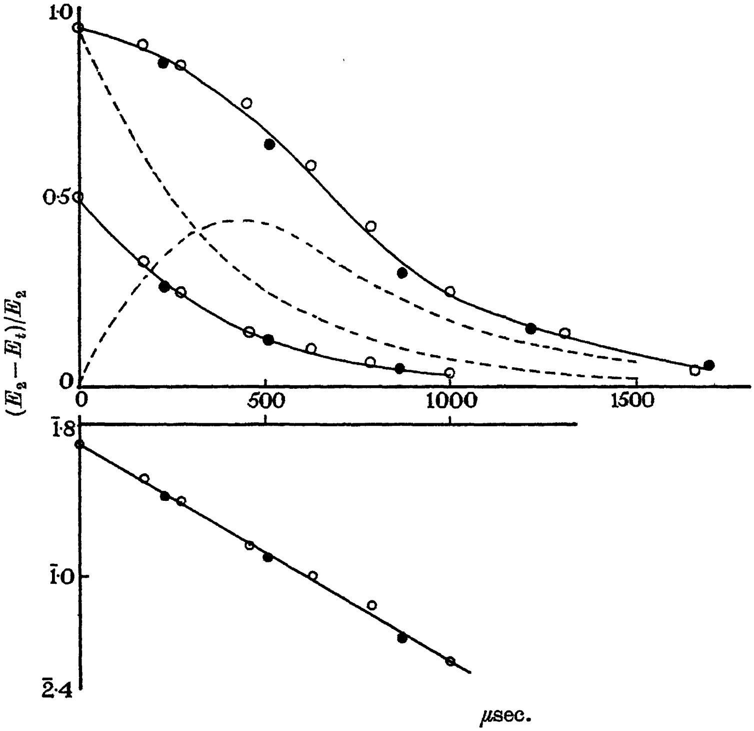

FIG. 3—"Local potential" and "local response". Experiment of 18 March 1937. 6° C. Excitability curves as in fig. 2, with $E_1 = 0{\cdot}95$ and 0·5. Analysis of upper curve shown in broken lines. Lower figure: results with $E_1 = 0{\cdot}5$ plotted semilogarithmically; the slope of this line gives $k = 380$ μsec.

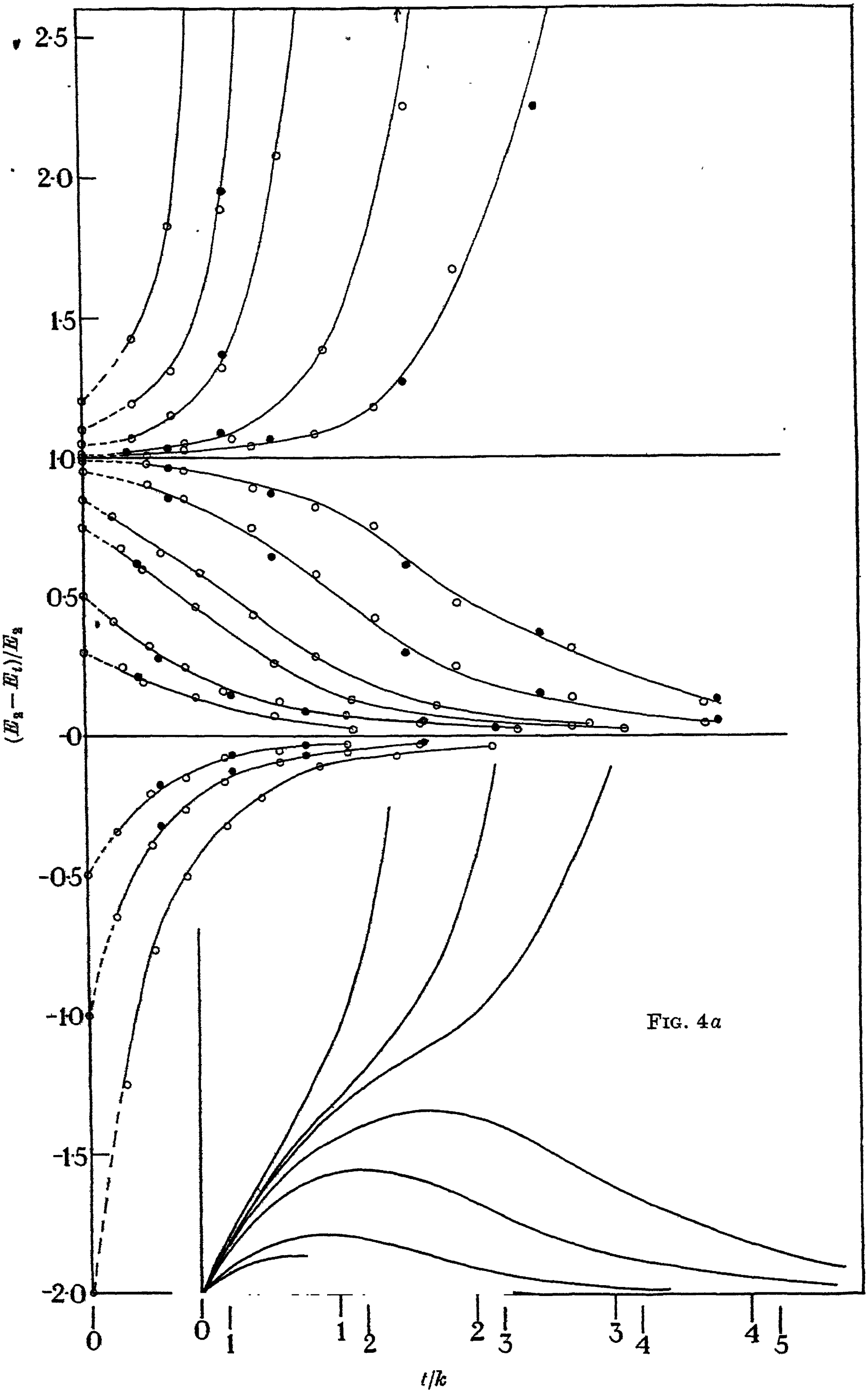

Fig. 4

As a matter of routine, the curve for $E_1 = 0{\cdot}5$ was used in most cases as the "standard curve" of decaying "local potential", serving as analyser of the others. This procedure is only approximate, since even with $E_1 = 0{\cdot}5$ a slight local response may be present; for reasons of experimental accuracy, however, this is preferable to the use of still weaker conditioning shocks.

One obtains then curves as in fig. 4, showing the size and time relations of the supposed local response to various subthreshold shocks.

Some interesting details require comment. The local response increases in amplitude, as well as duration, and its rising phase becomes longer, as the strength of the stimulus increases. Plotting the peak value of the local response (actually the maximum percentage of threshold change produced by it) against intensity of conditioning shock, one finds a relation (fig. 5) showing a "threshold" and then rising at an accelerating rate, till the local response reaches propagating size.

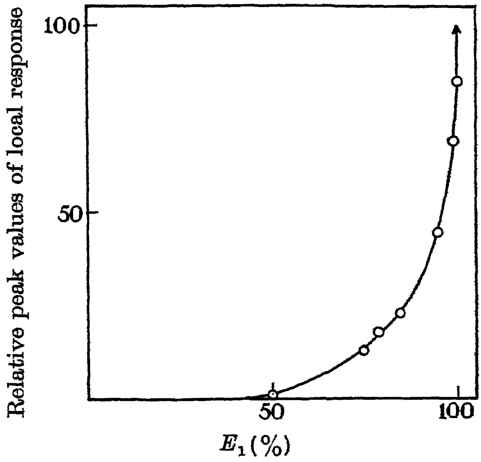

FIG. 5—Relation between maximum height of local response and strength of stimulus. Ordinate: maximum percentage change of threshold, due to local response. (With $E_1 = 1{\cdot}0$, the point of inflexion has been taken as the "critical maximum" of local response.) Abscissa: percentage strength of conditioning shock. Most of the data are mean values of several experiments: number of experiments for each value (successively from above): 9, 11, 22, 2, 1, 5, 6.

FIG. 4—Time course of excitatory disturbance and of local response (cf. Katz 1937, fig. 1). The data are taken from a number of typical experiments, performed on different preparations. Ordinate as in fig. 2. Abscissa: t/k, where k is derived from the curve with $E_1 = 0{\cdot}5$ in each experiment. E_1, successively from above: 1·2, 1·1, 1·05, 1·01, 1·0; 0·99, 0·95, 0·85, 0·75, 0·5, 0·3; −0·5, −1·0, −2·0.

FIG. 4*a* (Inset)—Shape of local response, obtained by subtracting the exponentially decaying "local potential" ($y = E_1 e^{-t/k}$) from the respective curves in fig. 4. E_1, successively from above: 1·05, 1·01, 1·0; 0·99, 0·95, 0·85, 0·75.

This relation has a very gradual and slow initial rise from its base-line. There is clearly a limiting intensity below which no local response at all can be detected; the threshold, however, for local response is not sharp, and it is difficult to say what strength of stimulus would just excite "at a point" only.

On the other hand, the relation becomes steep near to threshold for propagated response, which involves certain experimental difficulties when working with conditioning shocks only 1 % below, or equal to, threshold strength.

Slight spontaneous threshold changes, or inaccuracies in the adjustment of E_1, may then cause an appreciable "scatter", and actually this could not always be avoided. Such "random scatter", however, if present, affected only the later (falling) portion of the excitability curve, while its initial part was accurately determined, and, in any case, was not large enough to disguise the general shape of the resulting relation.

The precautions taken to reduce these inaccuracies were as follows: (i) the size of the threshold index was carefully kept constant; usually a minimal, single or repetitive, response of the gastrocnemius, detectable by the movement of an attached lever, during stimulation periods of a few seconds, was taken as index; (ii) by means of the variable Sullivan condenser, a quick and fine adjustment of E_1 was possible. Threshold determinations with E_1 alone, and with E_1+E_t, were repeated alternately, in order to control spontaneous threshold changes.

Another possible error must be considered. It is well known (see e.g. Hill, Katz and Solandt 1936) that subliminal pulses repeated at a rather low frequency may gradually become effective, due to some slow process of local "facilitation". Such subliminal facilitation was often observed, and then the respective thresholds of E_1 and E_2, determined separately, might possibly be lowered by some per cent when both shocks are applied together. Under certain conditions, therefore (namely, when conditioning and test shocks are both close to threshold), E_1 might conceivably become effective owing to additional facilitation caused by the preceding test shock, or E_t might be lowered due to additional facilitation by E_1. The effect would be to raise somewhat the final level to which the excitability curves tend to return. Actually, intervals not greater than about 2 msec. were used, since the phenomena investigated occur within this period. By this time (low temperature) the effect of $E_1=0{\cdot}95$ had usually decayed to about 5 %, that of $E_1=0{\cdot}99$ to 10–15 % of threshold, both curves still tending to fall (figs. 3, 4). If the decay is quickened by the use of a small interpolar distance (p. 260 below) the residual effect of $E_1=0{\cdot}99$ may have almost completely disappeared after this interval. It is unlikely, therefore, that any serious error is caused by mutual facilitation of conditioning and test shocks, even when both stimuli are close to threshold.

The presence of the local response explains another phenomenon, which has recently been described also by Fabre (1935), viz. the "non-linear" increase of the excitatory effect with strength of stimulus (fig. 6), if tested at some given time after its start. Fig. 4 shows that while the immediate excitatory effects of short shocks are approximately proportional to the applied charge, this relation becomes more and more curved as time goes on and, at last, the residual effects of weak shocks may be zero, while with stronger shocks a considerable remainder, due to a local response, is still left.

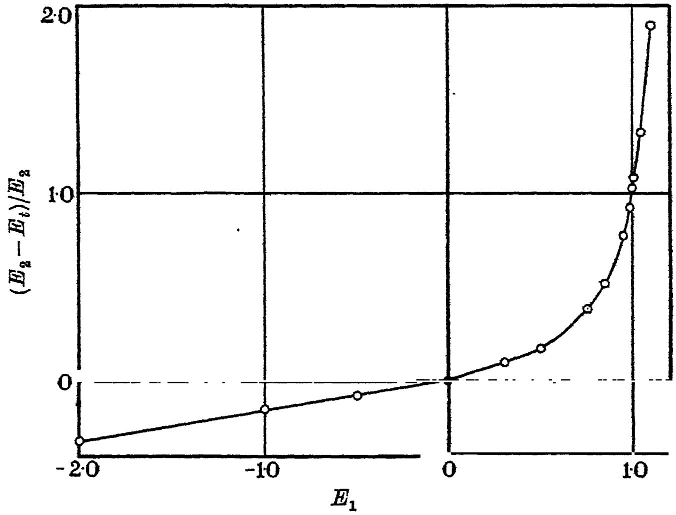

FIG. 6—Relation between size of excitatory disturbance at a given moment and strength of conditioning shock (cf. Fabre's (1935) "non-linear" increase of excitability). Data are taken from fig. 4, at interval $t=k$. Ordinate: $(E_2-E_t)/E_2$. Abscissa: E_1. Note the discontinuity of the slope of this relation at the origin, corresponding to a somewhat different time constant at cathode and anode (see p. 257).

Here again, it is difficult to say with what current strength the "non-linearity", i.e. the increasing growth of excitability, starts. Even between $E_1=0{\cdot}3$ and $0{\cdot}5$ the relation may bend up slightly.

The Graduation of the Local Response—It is obvious, as already pointed out by Adrian (1933), that the size of a non-conducted response, localized in the stimulated region, would depend upon the strength of the applied stimulus. The various factors involved may be appreciated best if we consider the local response as a secondary "local potential", built up not by an applied current, but by "local action currents" flowing along the outside of the fibre from the resting to the active region (Hodgkin 1937a, fig. 9). One can then reconstruct, by means of a graphical analysis (Rushton

1937*b*), the form and relative strength of an equivalent stimulating current from the observed excitability changes: the result is shown in fig. 7. It suggests that the local action current is equivalent to a short electric current pulse of abrupt start and gradual disappearance, which gradually

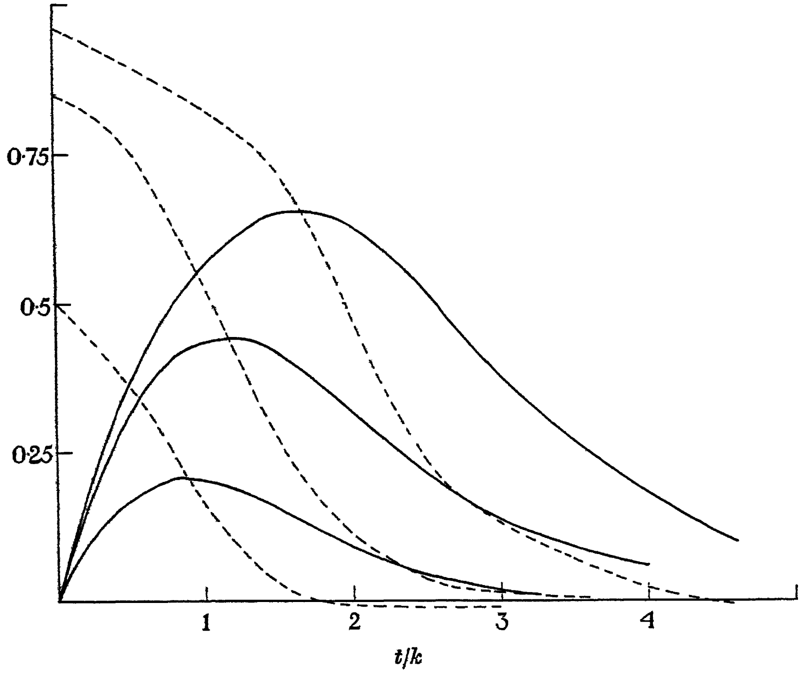

FIG. 7—Local action current, expressed as equivalent applied current, producing the effect shown in fig. 4*a*. Applying Rushton's (1937*b*) graphical analysis, the form of an electric stimulus has been traced, the effect of which, according to Hill's (1936*a*) theory, would be identical with the obtained local response. Full lines: local responses with $E_1 = 0{\cdot}99$, 0·95, 0·85. Broken lines: electric pulse by analysis. Ordinate: (*a*) full lines, as in fig. 3. (*b*) broken lines, *bkI*, where *b* is a constant, and *I* proportional to the strength of a stimulating current which, when applied through the same electrodes, would produce a "local potential" as shown by the full lines.

builds up a "local action potential", just as an applied slow condenser discharge would gradually build up a "local potential" (Hill 1936*a*).

As E_1 increases, the strength and duration of this secondary, intrinsic, stimulus also increases. The increment of local response with strength of applied stimulus (fig. 5) is, therefore, caused by at least two mutually assisting factors: (i) greater strength of the local action currents, (ii) activity being maintained for a longer time.

It remains to be asked whether the increase in strength of the local action *currents* is due entirely to the increasing length of the excited region (Rushton 1937*c*), or also to graded activity at any point.

It is implicitly assumed by most excitation theories that the local change (reduction or abolition of the resting potential), associated with activity, takes place suddenly and completely, as soon as a critical level of depolarization, at any point, is reached. It should be borne in mind, however, that the whole of the evidence for the all-or-nothing law and for the existence of a "critical threshold" refer to the propagated disturbance only and not to excitation at a given point. It is possible that local activity is not an all-or-none response to the applied stimulus, but increases gradually with increase of the latter.

Group (*iii*)—Excitability curves obtained with various anodic conditioning shocks ($E_1 = -0\cdot5$ to $-2\cdot0$) are all similar in shape: the return of the "local potential" to its base-line is approximately exponential and never shows a "hump" as with the stronger cathodic shocks (figs. 2 and 4).

This provides good confirmatory evidence that the "humps" on the cathodic side are really due to response and not, for example, to a physical distortion of the local excitatory currents by some interstitial tissue membrane (see Blair and Erlanger 1936). In the latter case, the deviation from the theoretical exponential decay not only should be proportional to the applied current strength, but also should be observed symmetrically at cathode and anode.

The form of the anodic excitability curves was often not exactly exponential, showing a relatively too rapid initial decay and then tailing off more slowly; the time factor could then be determined only approximately. Furthermore, a slight "disproportion" could often be detected when comparing the effects of different anodic shocks at a given interval: that is to say, doubling the ordinate distance for $E_1 = -0\cdot5$ gave a slightly larger value than actually found with $E_1 = -1\cdot0$, and doubling this gave slightly more than observed with $E_1 = -2\cdot0$. This effect, however, was small and possibly due to experimental error.

Figs. 2 and 4, and Table II show that the decay of the "local potential" was consistently quicker with anodic than with cathodic shocks. This

TABLE II—TIME FACTOR OF "LOCAL POTENTIAL" AT ANODE AND CATHODE

($E_1 = 0\cdot5$ and $-0\cdot5$ to $-1\cdot0$)

Temp. ° C.	3·9	3·0	4·3	3·9	6·0	5·0	5·3	7·0	6·9	5·5	Mean 5° C.
k cath.	390	425	460	460	350	285	425	400	240	440	385 μsec.
k an.	270	300	270	290	250	150	250	265	175	340	255 μsec.

difference may simply be due to stimulation of different fibres by the test shock after cathodic and anodic conditioning, though other factors may also be involved. If, for example, there are a number of fibres with approximately equal threshold (for a short shock), but different time constants k, one would have, for two opposite shocks (say $E_1 = +0{\cdot}5$ and $-0{\cdot}5$), a distribution of excitability curves as shown in fig. 8. Since the test shocks select those curves with the smallest ordinate distances from the threshold, one would necessarily detect the "slowest" fibres at the cathode and the "quickest" at the anode. This may be decided by working with single fibre responses.

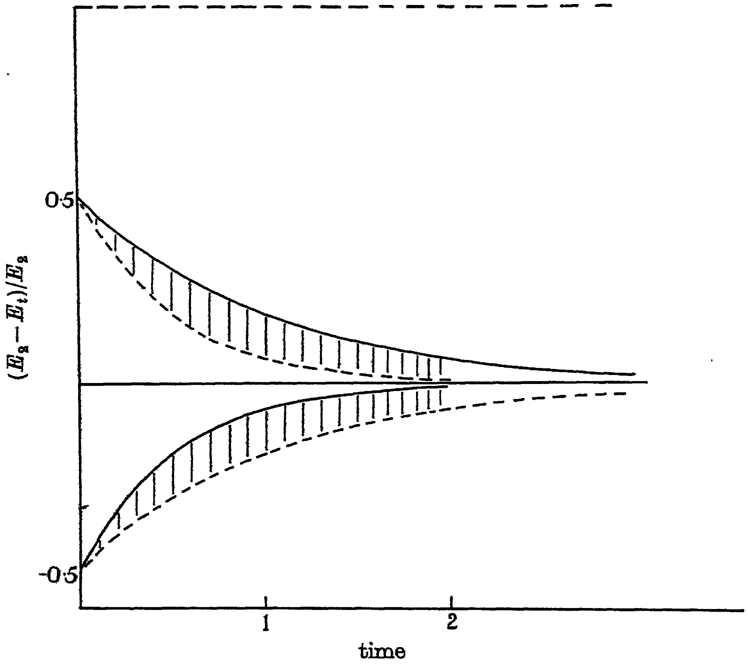

Fig. 8—Diagram illustrating the possible influence of various fibres with different time constants, on the excitability curves observed at anode and cathode.

Group (*iv*)—The experiment can be continued to superthreshold conditioning shocks ($E_1 > 1{\cdot}0$), since it is possible then, as Blair and Erlanger (1936) have shown (cf. also Dittler 1925; Colle 1934), to prevent propagation by applying anodic test shocks, even after a considerable interval. During this period, therefore, the time course of the "total excitatory disturbance" was tested, by determining, at any moment, that strength of an opposing shock which just allowed propagation to occur. The resulting excitability curves (fig. 4) again represent the sum of (i) the decaying "local potential", and (ii) a local response of the nerve, which gradually evolves to propagating size. The curves rise at an accelerating rate, which increases with the strength of the conditioning shock.

These curves have a special interest, since the amount of anodic charge, required at any moment to suppress conduction, gives us a direct measure of the initial development of the "safety margin" (Hodgkin 1937*a*; Rushton 1937*c*), with which the "full-grown" impulse propagates itself. If the local response, building up gradually and supported to some extent by the decaying "local potential", becomes strong enough to excite adjacent points, then their activity will add and contribute to an increasingly rapid growth of the "safety margin" up to its normal size.

The shape of the local response and its gradual transition into a propagating impulse is traced again by subtracting the "local potential" which subsides with time constant k, from the excitability curve (fig. 4*a*). It is particularly instructive to compare a response which is just able to propagate ($E_1 = 1{\cdot}0$) with a local response just failing to do so ($E_1 = 0{\cdot}99$). The initial development is very similar in both cases; then, however, the latter passes through a maximum and gradually declines, while the former passes through a point of inflexion after which it rapidly grows up.

With $E_1 = 1{\cdot}0$, the inflexion was observed, on the average, at an about 25 % higher level, and at nearly the same time (at 5° C., 620 and 610 μsec. respectively, though with differences of ± 100 μsec.) as the crest with $E_1 = 0{\cdot}99$. The inflexion occurs the earlier the stronger the conditioning shock; it is then difficult, however, to determine its position, because the curves become very steep from their beginning.

The fact that a given "safety margin" is reached earlier the stronger the applied stimulus, is demonstrated more clearly by fixing the strength of the opposing shock, and finding, for any interval, the propagating strength of the superthreshold "conditioning" shock (fig. 9, cf. Blair and Erlanger 1936). The result illustrates again that, with shocks just above threshold, a slight variation in strength of stimulus causes a very marked change in the rate at which a propagating impulse evolves.

Experiments on Non-Medullated Nerve—It was of interest to repeat these experiments on non-medullated nerve. Limb nerve-muscle preparations of *Carcinus maenas* (Pantin 1934; Katz 1936*b*) were used, and the response of the flexor muscle of the dactylus taken as index. In this way one is able to reduce, or even to eliminate, the effects of stimulating different fibres, since crab-limb muscles are known to consist of very few motor units, possibly even of only one. The nerve was dissected out along the meropodite, and placed on wooden strips soaked in crab-Ringer's solution and connected to calomel half-cells. Repetitive double shocks, at 20/sec. were applied, as described above (2000ω shunt, 5000ω series resistance, F_1 and F_2 as before). The response of this muscle, at 20° C., to 20 nerve impulses per sec. is very weak; by suitable arrangements, however (viz. observing the movement of a light lever, attached to the dactylus, against a fine mark), it could be used as threshold index in most preparations.

The results of excitability measurements are, in all essential points, very similar to those recorded on frog's medullated nerve. The duration of the excitatory disturbance is, for the same temperature, greater than in frog's sciatics, though the differences are not very great: the time factor of the "local potential", for example, averaged, in twenty-two experiments at 21·5° C., 250 μsec. (comparing with 180 μsec. in frogs). With stimuli near threshold, distinct "humps" occurred, while no such divergences were observed on the anodic side. In contrast, however, with frog nerves no significant difference was found, between anode and cathode, in the time factor of the "local potential": this supports the suggestion (p. 257) that this difference is only apparent and merely due to taking the index of response from different fibres.

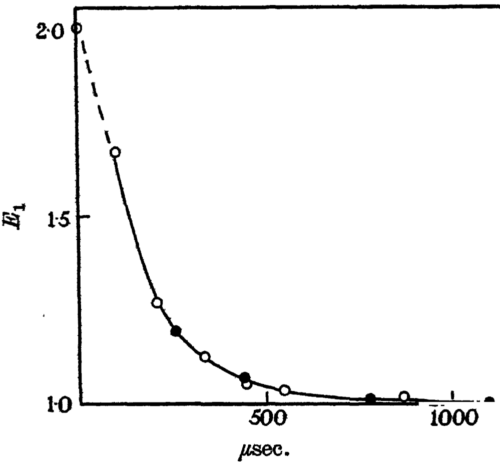

FIG. 9—Relation between strength of superthreshold shock and time required for reaching a given "safety margin". The "safety margin" is fixed by making the strength of the second shock $E_2 = -1·0$. Experiment of 9 March 1937. Temp. 4·5° C. Ordinate: strength of E_1 required. Abscissa: time, at which $E_2 = -1·0$ no longer prevents propagation.

The Effect of Small Interpolar Length—Information on the effect of small interelectrode distance was derived mainly from three experiments at low temperature, a summary of which is given in Table III.

TABLE III—EFFECT OF INTERPOLAR LENGTH

	Long distance				Short distance			
			Local response with $E_1 = 0{\cdot}99$				Local response with $E_1 = 0{\cdot}99$	
Temp. ° C.	Dist. mm.	k µsec.	Maximum height (% of threshold)	Crest at t µsec.	Dist. mm.	k µsec.	Maximum height (% of threshold)	Crest at t µsec.
4	26	400	71	700	1	160	81	450
3	29	310	75	650	1·5	170	82	400
3·5	26	305	70	550	2	160	84	450

Reducing the interpolar length (e.g. from 20 to 2 mm.) modifies the results in various respects. The time constant of the decaying "local potential" ($E_1 = 0{\cdot}5$) is reduced to about the same extent as the time constant k of the strength-duration curve. ("Rheobase" and "short shock threshold" were measured in these experiments so that $k (= Q_0/I_0$, Hill 1936*a*) was known.) "Overlapping" of cathodic and anodic region with small interpolar distance (Rushton 1934) and a continued lateral spread of the subsiding "local potential" would explain (i) the reduced efficacy of the stimulus, and (ii) the quicker decay of its excitatory effect.

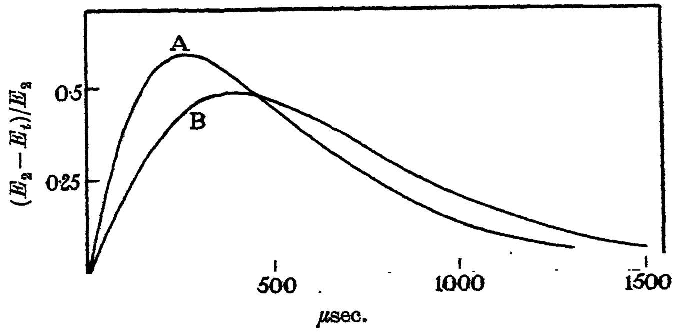

FIG. 10—Local responses with $E_1 = 0{\cdot}95$, obtained with small (A) and large (B) interpolar length. Experiment of 23 March 1937. Temp. 4° C.

Shape and size of the local responses (fig. 10) are similar to those found with long distance, though differing in some interesting detail. The duration of the rising phase is shorter, the rate of rise quicker, and the peak value larger than with long distance. It seems reasonable to suppose that, with small interpolar length, a larger local response is required to set up an impulse, since the "local potential" disappears more rapidly and, therefore, gives less support to the developing action potential.

At room temperature, similar phenomena but occurring quicker were observed. An interesting effect was found when applying anodic conditioning shocks. After a very short time, the residual effect of the conditioning shock was reversed, i.e. applying two successive shocks of *opposite* direction through a small interpolar length produced a distinct local *summation*. Actually, this phenomenon might have been expected from the previous results. The "local potential", at each electrode, falls rapidly to a small value, while a local response evolves at the cathode, which, though unable to excite, raises the excitability of the adjacent region. The second shock, therefore, tests actually the combined effects of (i) a disappearing anodic "local potential" and (ii) the spread of a very close local response, the centre of which is only 1–2 mm. away. The supposed interaction of these two opposite processes is illustrated in fig. 11.

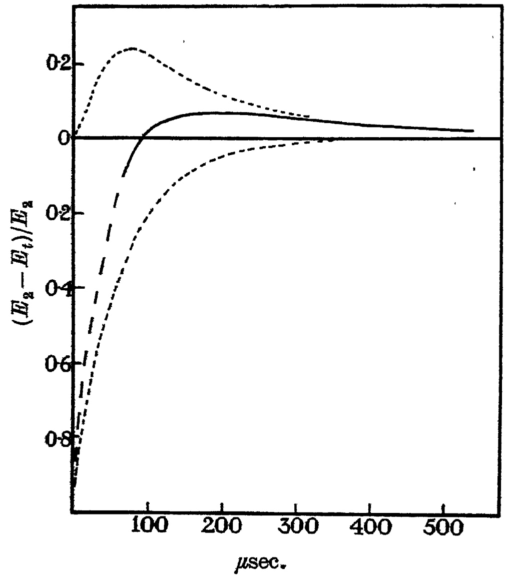

FIG. 11—Interference of two opposing phenomena, when an anodic is followed by a cathodic shock through a small interelectrode distance. Full line: observed excitability curve with $E_1 = -0{\cdot}95$ (room temperature). Broken lines: lower curve, "local potential" produced by E_1 at the "tested point". Upper curve: local response, caused by E_1 at the opposite electrode, spreading over to the "tested point".

These, however, may not be the only factors involved. If the difference described above in the decay of "local potential" at cathode and anode be genuine, and not merely due to different fibres, it may account also for spatial and temporal summation with shocks alternating in direction (cf. also § II).

II—ACCUMULATING EFFECTS OF LOCAL RESPONSES DURING ALTERNATING HIGH-FREQUENCY STIMULATION

The preceding results allow us to predict, at least qualitatively, what will happen when a battery of short alternating shocks is sent through a nerve at a sufficiently high frequency.

(*i*) *Summation*—Although the small local response following the first shock may be quite insufficient to propagate, it may sum with those produced by subsequent shocks and may soon become strong enough to set up an impulse. The threshold intensity, therefore, for "critical propagation" should be lower for high-frequency stimulation than for a single shock.

(*ii*) *Secondary Threshold Changes*—A frequent succession of local action currents may be expected to produce slow after-effects (post-cathodal depression, accommodation, electrotonic excitability changes), very similar to those of a subliminal constant current or repetitive one-way pulses (Hill 1936*a*).

Such predictions, of course, cannot be tested with high-frequency one-way discharges, because the summation of direct stimulating effects (see Bugnard and Hill 1935; Hill 1936*a*) would then seriously interfere with, and perhaps altogether disguise, the phenomena sought. The proper method, however, would be the application of alternating high-frequency shocks or sine-wave currents, which, according to the classical theories, produce merely alternating changes in the "local potential", but no local summation or cathodal after-effects.

If, therefore, the predicted "one-way" phenomena can be observed with high-frequency alternating stimulation, one may suspect them to be due to the small local action currents adding to the excitatory effect of the cathodic half-cycles.

Summation of Excitatory Effects with High-frequency Alternating Current—The fact that threshold excitation by high-frequency alternating current involves the summing effects of many cycles was discovered some time ago by Gildemeister (1930). Later, it was found by Hill, Katz and Solandt (1936) that, at high frequency, the observed threshold/frequency relation falls considerably below Hill's theoretical curve. This divergence was the actual starting-point of the present research. The results of a careful study from various quantitative aspects suggested that, with threshold stimulation at a high frequency (3000–10,000/sec. at 20° C.), the total excitatory effect of each cathodic half-cycle is greater than the depressing action of the anodic half-cycle. Due presumably to the intervention of this enhancing factor, the threshold is lowered to about one-half of Hill's theoretical value; incidentally, therefore, the optimum initial effect of a high-frequency alternating current (Hill 1936*a*) is no greater, or not much greater, than its "steady" effect.

Reference to methods and experimental details must be left to a later report. At first I thought (cf. Gildemeister 1930) that some "rectification" of the "local potential" at high frequency might explain the divergences from theory. This view was abandoned during the course of the later experiments, which have been described in this paper. Primarily, I owe the present concept to a discussion, at that stage, with Dr. Rushton and Mr. Hodgkin, who were just working out the theoretical aspects of the present problem.

Spatial Summation of Local Response with Small Interelectrode Distance—A particularly significant effect can be predicted if high-frequency alternating current is applied to a small interpolar length of nerve. It has been shown already that two alternate shocks following each other at a short interval give a local "summation" effect if the electrodes are only 1 or 2 mm. apart. A local response at one electrode raises the excitability at the other electrode if their distance is small.

If such alternate stimuli are repeated at a high enough frequency, the local responses from both electrodes, growing simultaneously during a series of cycles, will assist each other in setting up a propagating impulse. This is best illustrated by the fact that the threshold difference between large and small interelectrode distance, found with a single short shock, may be completely cancelled or even reversed, when the same shocks are applied in a high-frequency alternating succession. This will be described in a later paper.

Catelectrotonus and Accommodation as a Consequence of Local Activity—The occurrence of local action potentials is confirmed by the fact that subthreshold alternating currents at high frequency (e.g. 5000/sec. at 20° C.) produce definite postcathodal effects (catelectrotonic excitability changes, accommodation), detectable only above a certain current strength. The experimental conditions must be suitably chosen. At room temperature, the "one-way" effects of alternating current become marked above 1000/sec.; an appropriate frequency, at which these phenomena can readily be studied, is 5000/sec.

A subliminal prolonged alternating current at this frequency causes after-changes in excitability, quite similar to those produced by a subthreshold constant current. The latter changes, as is well known, depend largely, in size and direction, upon the experimental conditions: e.g. nerves soaked in a K-rich solution give an intense threshold rise due to the summing effects of accommodation and catelectrotonic depression (Werigo 1901; Chweitzer 1935); in Ca-rich nerves, on the contrary, the catelectrotonic threshold *lowering* (with weak currents) may cancel or even overbalance the accommodative effect, and in normal nerves, intermediate phenomena are observed (see fig. 12). It is best, therefore, to employ K-nerves, in order to produce, even with relatively weak stimuli, a conspicuous effect.

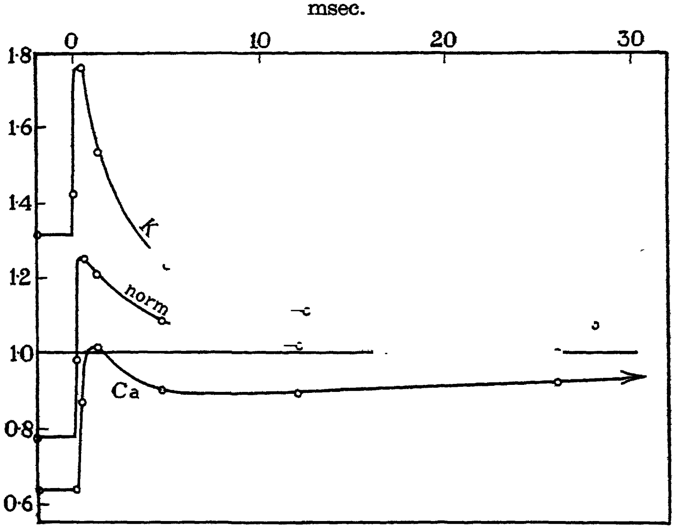

FIG. 12—Variation of post-cathodal effects, produced by K and Ca-rich solution. After-effect of constant current pulse (10 % below threshold) tested at various time intervals by a short shock. Ordinate: test shock threshold/resting threshold. Abscissa: time interval after break of current pulse. Three curves (successively from above) on K-rich, normal, Ca-rich nerves.

Time Course of "Post-cathodal" Depression—The time course of the excitability changes following a prolonged alternating current, or a constant current pulse, was tested by short shocks applied at various intervals after the conditioning stimulus, to a nerve which had been soaked for 1 hr. in a K-rich solution (10 times normal).

For this purpose the Downing commutator was used, rotating at 2 rev./sec.: the duration of each conditioning pulse was then about 0·25 sec. The experiment was made first with alternating current, then with an appropriate subrheobasic constant current, and finally, with alternating current again.

It will be shown below, that, in K-nerves, a slowly increasing alternating current may not excite, even if its final strength is far above ordinary threshold. Use was made of this observation in some experiments, in order to obtain larger after-effects: a slow anodic condenser discharge was applied, starting simultaneously with the alternating current pulse, so that the latter reached its full stimulating effect only gradually. In other cases, however, the strength of the conditioning alternating current was made just subliminal.

When the observed test shock threshold is plotted against the time interval from the end of the alternating or the constant current, two curves are obtained of similar time course. In some experiments the curves coincided completely, though this was not the rule: usually, the decay of the alternating current effect was somewhat slower. Fig. 13 represents the mean result of sixteen experiments, each of which gave a similarly smooth curve, though varying to some extent in size and time relation. In round numbers, the after-effect of a just subliminal alternating current was comparable to the post-cathodal depression of a constant current pulse of rather more than half-rheobasic strength.

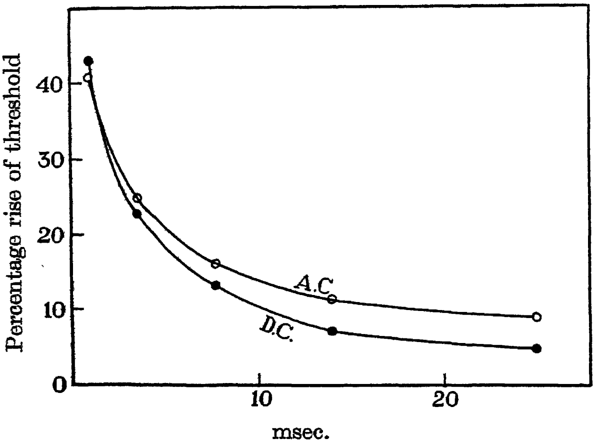

Fig. 13—The decay of "post-cathodal" depression following constant current and alternating current stimuli. Mean of sixteen experiments, made on K-rich nerves. Percentage rise of threshold plotted against time interval after break of conditioning stimulus. Hollow circles: conditioning stimulus alternating current at 5000/sec. Full circles: conditioning stimulus subrheobasic constant current. For further explanations see text.

The curves, especially that with alternating current, fall to a level, higher than the "resting" base-line: this is probably due to protracted electrotonic threshold changes (see fig. 12) which, during the resting interval of 0·25 sec. between pulses, would not completely disappear.

The Relation between Current Strength and Excitability Change—It is natural to suppose that the "post-cathodal" depression produced by alternating current is due not to the applied stimulus, but to local action currents which do not alternate in direction. This suggestion would be invalid if the after-effect were proportional to the applied current strength; it would gain strong support if there were a "threshold" intensity below which no such after-effects are found.

The simplest way of investigating this question is to apply long-continued alternating currents of various intensities and to increase them suddenly up to threshold. This was done by operating two Morse keys, the first breaking a short-circuit across the nerve, the second short-circuiting a variable series resistance. The regular appearance of a muscle twitch was taken as threshold index in order to avoid initial "random phase" effects (Hill 1936*a*). Thus, the alternating current increased in two steps with an interval of about 1 sec.

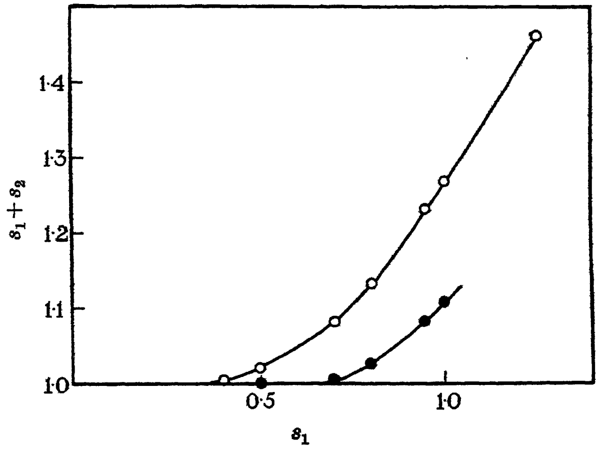

FIG. 14—Relation between size of "accommodative threshold rise" and strength of alternating current stimulus. The "accommodating" effect of a high-frequency alternating current (5000/sec.) is tested by increasing the stimulus in two steps (s_1 and s_2), the first lasting about 1 sec., the second suddenly reaching required threshold strength. Ordinate: effective final current strength. Abscissa: strength of first step. Full circles: normal nerves. Hollow circles: nerves soaked in 10 times K-Ringer.

The mean result of eight experiments (four on normal, four on K-nerves) is shown in fig. 14, where the total current strength required is plotted against the intensity of the first step. The observed relation has a definite "threshold" below which no depressing effect of the alternating can be detected. This threshold is considerably lowered and the strength of the depression increased by K.

This result seemed satisfactory, but it was necessary to get comparable results with constant current. For this purpose, the after-effects of subthreshold alternating currents (5000/sec.) and of subthreshold constant currents of various intensities were tested, at a given interval, by condenser shocks, using Downing's commutator as above. A time interval of about 1 msec. was chosen, after which, at room temperature, the "local potential" has decayed to a very small value, so that the random phase at which the alternating current breaks does not matter, while the post-cathodal effect is still strong.

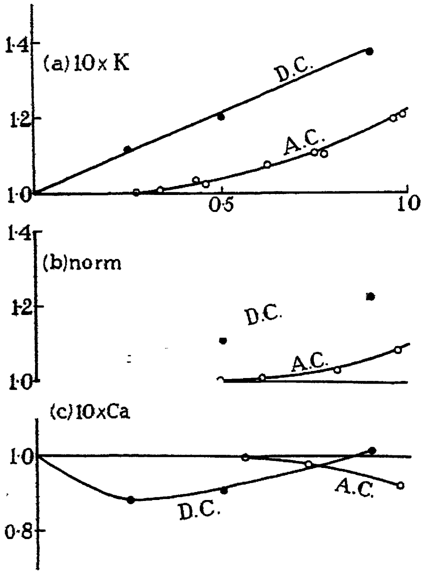

FIG. 15—Comparison of post-cathodal effects produced by (i) alternating current (5000/sec. hollow circles) and (ii) constant current (full circles) under different "electrotonic" conditions. (*a*) 10 times K, (*b*) normal, (*c*) 10 times Ca. Excitability tested at a given interval (about 1 msec.) after break of current pulse. Ordinate as in fig. 12. Abscissa: strength of conditioning stimulus as a fraction of threshold strength.

Typical results are shown in fig. 15. The post-cathodal threshold changes vary in size and direction, depending upon the electrotonic condition of the nerve (K, normal; Ca, cf. fig. 12). A corresponding variation is found in the after-effects of an alternating current. The latter again appears to be equivalent to the post-cathodal effect of a constant current of about half-threshold strength. The pertinent points are the obvious "non-linearity" and the existence of a "threshold" for the alternating current effect.

This "threshold" is sometimes rather low and its exact position doubtful, since the curves rise very gradually from the base-line. It should be realized, however, that there is no evidence that, nor any *a priori* reason why, a local response should have a sharp or definite threshold.

The alternating current experiments, as a whole, show a number of phenomena which cannot be explained on the basis of the classical theories, but are clearly consistent with the idea of a local response.

III—THE ELECTRIC SIGN OF LOCAL ACTIVITY

The experimental evidence for the existence in nerve of a non-conducted response cannot be regarded as convincing so long as a record of an accompanying local electromotive change is missing. The possibility of a local response has clearly been thought of before, but not accepted due to the failure to detect any electric sign (Kato 1934; Blair and Erlanger 1936). As Rushton (1937*c*), however, recently pointed out, the size of a non-conducted action potential may conceivably be so small that it escapes observation even with modern means of amplification. The difficulties in recording a local response to short shocks, with a quick, but insensitive oscillograph, are obvious: it is not only a matter of amplification: the shock "artefact" or electrotonus at the point of stimulation may be so great that, even if most of it were balanced, the unbalanced remainder might altogether disguise the phenomenon in question.

It is of great interest, however, that Auger and Fessard (1935), recording the electric activity of *Chara*, found distinct local action potentials with subliminal stimuli which, when reaching a certain size, grew to a propagating decrementless wave which obeyed the all-or-nothing relation. In these extremely slow plant organisms the influence of the shock artefact is much less serious than in medullated nerve.

(*Added in proof.*) Quite recently Hodgkin (1937*b*) obtained convincing records of local action potentials from isolated crustacean nerve fibres.

It seemed necessary, therefore, being primarily concerned with the *existence* of an electric sign, to sacrifice, for the time being, any attempt to record such details as can only be traced by oscillograph methods. The use of a sensitive galvanometer, therefore, was indicated, to sum up small rapid changes, following one another at high-frequency.

High-frequency alternating current, or alternating short condenser discharges at various frequencies (method, see Hill, Katz and Solandt 1936), were applied to the nerve, and local and conducted potential changes recorded as shown in fig. 16. By the combined use of short alternate pulses and a slow sensitive galvanometer disturbing phenomena such as electrotonus and various shock artefacts are eliminated (see below).

Usually the stimulating electrodes were placed at a site peripheral from the recording leads in order to avoid stimulating fibres (cut branches) which do not run through the whole length of nerve from which the record is taken. If possible, a uniform stretch of nerve was chosen for stimulation and local recording.

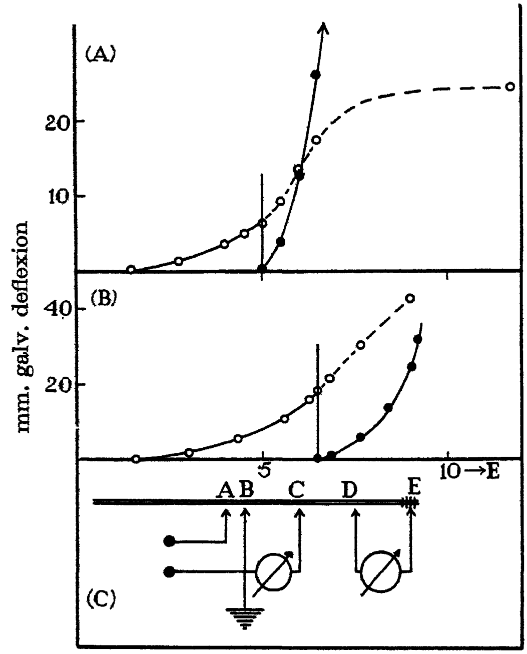

Fig. 16—Strength-response relation for local and conducted electric response, with high-frequency alternating stimulation. Hollow circles: local response, recorded at leads *BC*. The relation is drawn in full line up to "conduction threshold" only. Full circles: conducted response recorded at leads *DE*. (*A*) 1700 two-way condenser discharges per sec. Size of galvanometer deflexion plotted against relative strength of stimulus *E*. At $E = 11\cdot7$, the conducted response (not shown) has reached a size 10 times that of the local "negative variation". (*B*) Response to alternating current of 5000/sec. Plotted as in (*A*). Ordinate: galvanometer deflexion. Abscissa: strength of stimulating current. (*C*) Diagram of stimulating and recording leads.

The results, two typical examples of which are shown in fig. 16, were essentially the same for high-frequency alternating discharges or sine-wave currents. With subliminal stimulation, a small "negative variation" is invariably found, localized in the stimulated region.

This result might be due to various artefacts, such as rectification of ordinary current leak or of electrotonus, or blockage of impulses in some fibres damaged between the recording leads. The last possibility does not seriously apply to subliminal stimulation, since it is very unlikely that such blocked fibres, if present, would invariably have a lower, and often much lower, threshold than the rest of the nerve. Rectification of electrotonic currents, etc., would be more serious. The fact that the observed "strength/response" relation has a threshold and is not linear does not rule this possibility out entirely.

The initial rise of the relation in fig. 16 is again very gradual, and the position of its threshold is much less definite than that of propagated response. The same is seen in figs. 5 and 15, in which different aspects of the local response are shown. The present "strength-response" relation differs, however, from the other curves, since it involves not only the increase of local activity in each fibre, but also the increasing number of elements locally responding to an increasing current intensity.

A satisfactory test was obtained by narcotizing the nerve with alcohol. Soaking a nerve in 5–7 % alcohol/Ringer for about 20 min. regularly reduced the size of the local response with a given current intensity to about 10–25 % of its original size; stronger currents were required to produce the same effect. Conduction was in some cases abolished, in others not completely suppressed by this procedure. The effect was quickly reversible, the nerve might recover partly, even without washing, while set up in the—alcohol-free—paraffin chamber.

It was simpler and quicker, however, to leave the nerve on the electrodes and merely to place an alcohol-soaked strip of filter paper under the glass cover of the paraffin chamber. The local response disappeared then within 2–3 min. Soaking in Ringer's solution for 20–30 min. was required to restore local and conducted response, though complete recovery was not always obtained. By this procedure the resting potential was also reduced reversibly within a few minutes to a small value.

The fact that the observed "negative variation" produced by subthreshold stimuli (i) has a threshold and no direct proportionality to the applied current strength, (ii) is reversibly abolished by alcohol vapour, strongly suggests that it is a genuine response of the nerve and not an electric artefact.

On the other hand, it cannot be decided by the present method, whether the local response to high-frequency stimulation is due to rhythmic local activity, with a definite refractory period of its own, or to a more or less permanent depolarization, lasting as long as the stimulus is maintained.

The Effect of Frequency of Stimulation—When studying the relation between (i) size of local action current with a given strength of stimulus, and (ii) frequency of applied alternating short shocks, one finds that, at room temperature, the relation rises almost linearly, even up to 2000 two-way shocks per sec. Higher frequencies could not be employed; it seems likely, however, from measurements with alternating current excitation, that at 20° C. the local responses reach their "maximum summation" effect only at about 5000/sec. At low temperature (5° C.), the local response appears to attain its maximum at about 1000/sec. and to be the same at 2000/sec.

A surprising property of the local electric response is its steadiness during continued high-frequency stimulation: the local negative variation shows little or no decrease, while the conducted response, after reaching a maximum, falls to a considerably lower level (cf. Bugnard and Hill 1935). There is good evidence suggesting that the "inhibition" of nervous activity described by Bugnard and Hill (1935) and by Katz (1936*a*) for high-frequency alternating current stimulation, does not apply to the local response at all. Even K-nerves and nerves which have incompletely recovered from alcohol narcosis, give, at high frequency, a perfectly steady local response. When the alternating current is slowly increased, a K-nerve often shows no propagated disturbance at all, while the local response grows up to conspicuous size and, presumably by the strong catelectrotonic effect of its own, eventually *blocks* impulses, sent in from outside.

It may appear strange that such a strong depression should be produced by local activity without any sign of "inhibition" or "fatigue" in the active region itself. Considering, however, that the cathode of the "local circuit" is formed by the resting region in front of the excited part, it is quite natural to expect that cathodal depression (very strong in K-nerves) should occur further on, and not in the active region itself, which is excited by the applied alternating current.

The nature of the high-frequency inhibition was further investigated by comparing local and conducted response to strong alternating shocks. At low frequency, if the strength of the stimulus exceeds threshold the propagated response quickly "shoots up" while the local response soon stops increasing (fig. 16), and may even fall to a small value. It is difficult, however, to ascertain whether such records are not seriously affected by small possible differences in the size of the propagated wave at electrodes *B* and *C* (fig. 16), even when care is taken to use a uniform stretch of nerve far from the injured end. Actually, if all fibres produce regular propagated impulses, and provided the negative wave is identical and equally recorded at both leads, the local response should disappear, when the stimulus becomes maximal.

Striking and clear-cut results, however, are obtained at high frequency of alternating current. Confirming previous observations, it was found that as the strength of the stimulus rises, the conducted response reaches a maximum and then falls considerably (Katz 1936*a*). On the other hand, with maximal stimuli, as stimulation is continued, the propagated deflexion passes through a maximum and then progressively declines (Bugnard and Hill 1935).

In both cases, as the propagated response (at electrodes *DE*) fails, the local negative variation (at *BC*) increases, up to a steady level. In other words, the inhibition is due to a progressive failure of propagation only. The "complementary" effects of local and propagated response are clearly shown in fig. 17: this is, in fact, the same phenomenon, as previously described (Katz 1936*a*), viz. the decline of the strength/response relation is cancelled, when leading off close to the stimulating leads.

With continued stimulation, the local response (or rather the "localization" of the conducted response) tends towards a maximum at the same rate as the propagated disturbance declines to a small remainder (fig. 17). In the steady state the local activity is fully maintained for a very long time (more than 5 min.), and then decreases only very slowly. When the stimulus is withdrawn, the "local" deflexion falls quickly, with perhaps some delay in its final return, to a somewhat different "base-line", and often shows some prolonged after-positivity.

These experiments show that there is little or no failure of the applied alternating current to produce a local depolarization, but a progressive failure of this depolarization to propagate. These results, especially the K effect and the conduction failure reported in the last experiments, are particularly interesting, since they suggest a satisfactory explanation for the inhibitory effect of high-frequency alternating current: apparently this inhibition occurs, not because the alternating stimuli progressively fail to excite a sufficient region of nerve, but because the frequent and even strong local action currents progressively depress the resting parts farther on and therefore fail to propagate. Hill (1936*a*) suggested that, with high-frequency one-way stimulation, "cathodic depression" or "accommodation" soon intervenes and reduces the initial efficacy of the stimulus. With two-way and alternating current stimulation, the inhibitory phenomenon was not so easily understood.

It seems probable that, in the case of alternating current, cathodic one-way stimuli are actually formed by the "extrinsic"* local action currents, falling into the refractory period of the preceding propagated wave, and varying in strength, probably also in duration, with the intensity of the applied alternating current. In K-nerves, even weak cathodic currents cause an intense depression, normally, however, a progressive failure occurs only when the currents are strong and prolonged. This obviously applies to the case dealt with by Bugnard and Hill (1935) and by Katz (1936*a*).

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* "Extrinsic" in the sense adopted by Hodgkin (1937*a*), referring to the catelectrotonic spread of activity.

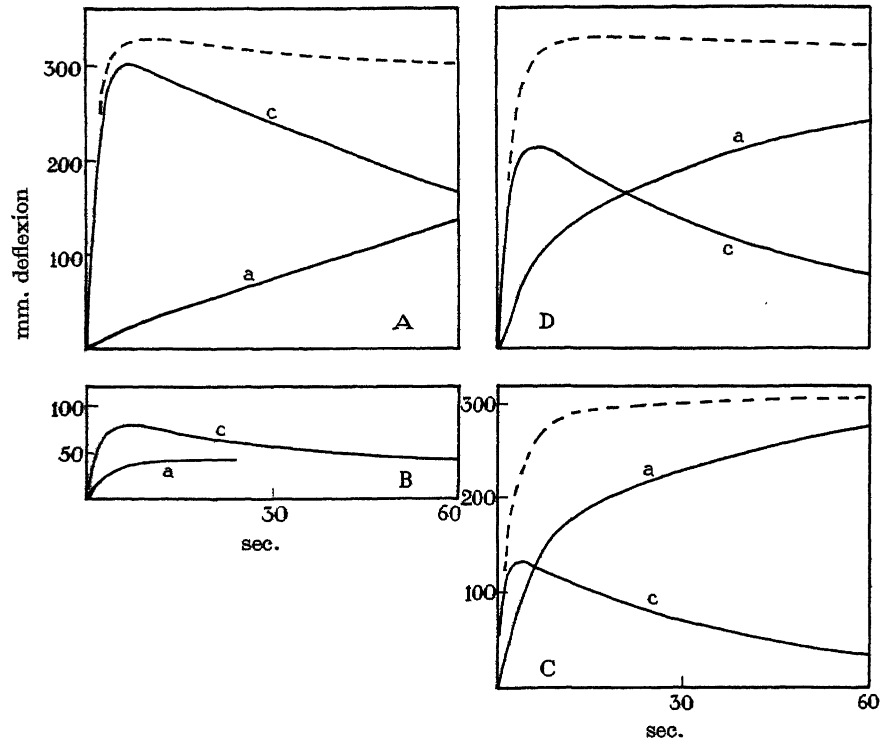

FIG. 17—Progressive "localization" of electric response during high-frequency alternating current stimulation of nerve. Galvanometer records, at "local" (*a*) and "conducted" (*c*) leads (cf. fig. 16) with prolonged high-frequency alternating currents (5000/sec.) of various intensities. (Relative strength: *A*, 14; *B*, 6·9; *C*, 19; *D*=*A*). The sum of local and conducted response is shown by broken lines. The progressive inhibition of electric response (Bugnard and Hill 1935) appears to be due to a process of progressive "localization" of the initially propagated action currents to the stimulated region. (Compare especially records *A* and *D*, obtained with same stimulus, at different stages of the experiment.)

In a recent paper, I suggested that an impulse, actually started off, may be outrun and blocked by the strong anelectrotonus of the following half-cycle. It seems difficult to dismiss this possibility, since a weak (refractory) impulse might easily be blocked by anelectrotonus before it has time to propagate over an appreciable length of nerve. That view was based, however, on a different interpretation of the observed persistence of activity in the stimulated region, even while and after the propagated response declined to a small final value. It was not realized then, that the observed phenomenon is probably a strong local response, due to the direct excitation of a large region of nerve, which, with prolonged stimulation, produces a strong catelectrotonic depression further on.

I am greatly indebted to Professor A. V. Hill for much helpful advice and continuous encouragement. The present work developed from his recent theory of excitation, which served as a most useful guide throughout a study intentionally of its imperfections. My thanks are also due to Mr. J. L. Parkinson for frequent and ready help, and to Mr. A. C. Downing for constructing the adjustable commutator. It is a pleasure also to thank Dr. H. Rosenberg, Dr. W. A. H. Rushton, Mr. A. L. Hodgkin and Mr. J. M. Ledingham for much suggestive discussion and criticism. Part of the expense of this work was defrayed by a grant from the Thomas Smythe Hughes Fund of London University.

SUMMARY

If the conduction of an impulse depends upon the excitation of each resting part by the activity of its adjacent region, a certain minimal length of active nerve is required initially to set up a propagated disturbance. Subthreshold stimuli, therefore, may, by exciting a small subliminal length of nerve, produce a transitory local response, the spread and size of which do not suffice to excite resting points further on.

Experimental evidence is given for the existence of this non-conducted response.

By excitability measurements with short double shocks, its presence, relative size and time relations are traced.

When the conditioning shocks are cathodic and weak, or anodic and of any strength, the decay of the "excitatory disturbance" follows a simple, approximately exponential relation.

When the strength of the conditioning shock approaches threshold, the time course of the local "excitatory disturbance" shows a "hump", superimposed on the exponential decay. This is attributed to the local response of a small region of nerve.

When superthreshold stimuli are applied, it is possible, using suppressing, anodic, test shocks to trace the initial growth of the new-born impulse, and its transition from local to propagating strength.

Various phenomena are observed with high-frequency alternating current stimulation (summation, accommodation, electrotonic excitability changes) which are inconsistent with the classical theories. Investigation of these phenomena shows that they can be attributed to local action currents, elicited by the cathodic half-cycles of the applied stimulus.

With a sensitive galvanometer and subthreshold alternating stimuli, a local negative electromotive change is found, having the properties of a non-conducted response.

REFERENCES

Adrian, E. D. 1933 *Ergebn. Physiol.* **35**, 744.
Auger, D. and Fessard, A. 1935 *C.R. Soc. Biol., Paris*, **118**, 1059.
Blair, E. A. and Erlanger, J. 1936 *Amer. J. Physiol.* **114**, 309.
Bogue, J. Y. and Rosenberg, H. 1934 *J. Physiol.* **82**, 353.
Bugnard, L. and Hill, A. V. 1935 *J. Physiol.* **83**, 394.
Chweitzer, A. 1935 *C.R. Soc. Biol., Paris*, **120**, 626.
Colle, J. 1934 *C.R. Soc. Biol., Paris*, **117**, 99.
Cremer, M. 1929 *Handb. norm. path. Physiol.* **9**, 244.
Dittler, R. 1925 *Z. Biol.* **83**, 29.
Erlanger, J. and Blair, E. A. 1931 *Amer. J. Physiol.* **99**, 108.
Fabre, Ph. 1935 *C.R. Soc. Biol., Paris*, **119**, 191.
Gasser, H. S. 1931 *Amer. J. Physiol.* **97**, 254.
— 1937 In Erlanger, J. and Gasser, H. S., "Electrical Signs of Nervous Activity." Philadelphia: Univ. of Pennsylvania Press.
Gildemeister, M. 1908 *Pflüg. Arch. ges. Physiol.* **124**, 447.
— 1930 *Ber. Sächs. Akad. Wiss.* **81**, 303.
Harris, D. T. and Rosenberg, H. 1935 *J. Physiol.* **84**, 11*P*.
Harris, D. T., Rosenberg, H. and Sager, O. 1936 *J. Physiol.* **86**, 4*P*.
Hermann, L. 1899 *Pflüg. Arch. ges. Physiol.* **75**, 574.
Hill, A. V. 1936*a* *Proc. Roy. Soc.* B, **119**, 305.
— 1936*b* *Proc. Roy. Soc.* B, **119**, 440.
Hill, A. V., Katz, B. and Solandt, D. Y. 1936 *Proc. Roy. Soc.* B, **121**, 74.
Hodgkin, A. L. 1937*a* *J. Physiol.* **90**, 183.
— 1937*b* *J. Physiol.* **91**, *P* (in the press).
Kato, G. 1934 "The Microphysiology of Nerve." Tokyo: Maruzen Co.
Katz, B. 1936*a* *J. Physiol.* **86**, 285.
— 1936*b* *J. Physiol.* **87**, 199.
— 1937 *J. Physiol.* **90**, 6*P*.
Lucas, K. 1910 *J. Physiol.* **39**, 461.
Monnier, A.-M. 1934 "L'excitation électrique des tissues." Paris: Hermann et Cie.
Pantin, C. F. A. 1934 *J. Exp. Biol.* **11**, 11.
Rashevsky, N. 1931 *J. Gen. Physiol.* **14**, 517.
Rushton, W. A. H. 1934 *J. Physiol.* **82**, 332.
— 1937*a* *J. Physiol.* **90**, 5*P*.
— 1937*b* *Proc. Roy. Soc.* B, **123**, 382.
— 1937*c* *Proc. Roy. Soc.* B, **124**, 210.
Werigo, B. 1901 *Pflüg. Arch. ges. Physiol.* **84**, 547.

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Reversible Oxidation of Chlorophyll

By E. Rabinowitch and J. Weiss

From the Sir William Ramsay Laboratories of Inorganic and Physical Chemistry, University College, London, W.C. 1

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In this paper some experiments are described showing that chlorophyll reacts with certain oxidizing agents—especially with *ferric salts*—forming a yellow product, from which it can be easily regained, for instance, by the action of *ferrous salts*.

Photoelectric measurements were made of the extinction curves of:

I. The methyl alcohol solutions of chlorophyll (both the two chlorophylls *a* and *b*, and the "natural" mixture of the two ethyl chlorophyllides).

II. The solutions of the same three substances after reaction with $FeCl_3$.

III. The same solutions "restored" by $FeCl_2$.

Solution (II) showed the complete absence of the characteristic red absorption band of chlorophyll, whereas in solution (III) this band was restored to its original shape.

Fluorescence of chlorophyll was shown to drop to 20–25% of its original value by addition of $FeCl_3$, and to reappear in its original strength by addition of $FeCl_2$.

These experiments are explained by the assumption of an equilibrium $\text{Chl} + \text{Fe}^{III} \rightleftharpoons \text{oxy-Chl} + \text{Fe}^{II}$, where oxy-Chl is an oxidation product which may be, for instance, a positive chlorophyll-ion, or a dehydrochlorophyll.

The oxidation theory of the reaction of chlorophyll with Fe^{III} is confirmed by a sensitive test for Fe^{II} in a mixture of chlorophyll with $FeCl_3$. The reversible character of the reaction is verified by the possibility of repeating the cycle after separating the "restored" chlorophyll from the iron salts.

It is further shown that chlorophyll is also restored in the solutions decolorized by $FeCl_3$ by keeping them for several hours in the dark at room temperature. This is due to the disappearance of $FeCl_3$, which apparently oxidizes the solvent (CH_3OH) in the presence of chlorophyll. Chlorophyll is thus able to act as an oxidation-reduction catalyst in the dark. The reaction of chlorophyll with ferric chloride is accelerated by illumination.

Further experiments described show the instability of the "oxychlorophyll" towards light and water, the influence of salts upon the equilibrium, the special effects obtained in the violet absorption band of the *b* component, and the analogous equilibria obtained in the systems Ce^{4+}—Ce^{3+}, Cu^{II}—Cu^{I}, and I_2—I^-.

(*The full paper is published in Proc. Roy. Soc. A*, **162**, 251.)

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Factors Influencing the Effectiveness of Administered Hormones

By R. Deanesly and A. S. Parkes, F.R.S.

(*Received* 9 *September* 1937)

[Plates 10–13]

Endocrine organs concerned in producing chronic effects in the normal body may be supposed to keep up a continuous supply of secretion so that the concentration of the particular hormone in the blood stream neither falls below the required threshold level nor rises sufficiently above it to lead to adverse effects. It has now become clear that the chief problem in substitution therapy with hormone preparations is so to arrange administration as to imitate the steady continuous activity of the normal glands and avoid alternation of deficiency and wasteful excess of the hormone. Thyroxin alone among the hormones appears to be fairly long-lived in the circulation, so that an excess at a particular time is not necessarily wasteful. On the other hand, the hormones of the gonads, anterior pituitary and adrenal cortex, as well as insulin, seem to be used, excreted or destroyed soon after entering the circulation. Quantitative and sometimes even qualitative results with these hormones have depended on the method of administration adopted in assay work.

In the case of oestrogenic preparations, from ovary or urine, attention was early directed to the variation in apparent activity due to the method of administration. The significant facts in this connexion have been recently reviewed (Parkes 1937). A constant total dose of oestrone, oestradiol or ovarian extract in either oily or aqueous solution, is much more effective if given as a series of small doses than if given as a single dose. The effect of divided dosage can be simulated by appropriate esterification of the hormone.

Analogous facts have had a considerable influence upon the course of research on the male hormones. The loss of effectiveness of both urine and testis extracts when highly purified led to the supposition that some inactive but synergic substance was necessary for full activity of the hormones and that this substance was fractionated away during purification of the active principles (David and others 1935). It is now known that both androsterone from urine and testosterone from testes are readily absorbed by the body, and that an injection in concentrated oil solution leads to temporary stimulation only and to wastage of any excess by destruction or excretion. It is possible therefore to level up the supply to the animal and increase the effectiveness of the total dose by decreasing the rate of absorption in various ways, by adding (or in the case of extracts not removing) inactive acidic fractions of urine and testes, by increasing the amount of oil solvent, or by mixing fatty acids with the oil solution. Analogy with oestrone led to the preparation of aliphatic esters of testosterone and to the demonstration by Miescher, Wettstein and Tschopp (1936) that the duration of action of the esters increased with increase in the number of carbon atoms in the acid chain. Testosterone formate, acetate and propionate showed, too, an increasing intensity of action as compared with the free hormone, owing presumably to decrease of wastage, but in the higher esters activity became so prolonged that intensity of effect was inevitably reduced. The propionate appears to give the optimum combination of intensity and duration of action, and is, in fact, more effective given once in 10 days than is a similar total dose of free hormone given in twenty injections, twice each day (Parkes 1936). In contrast to the increased effects shown by the aliphatic esters, the succinates of androsterone and androstanediol, water-soluble salts, are much less effective than the free hormones (Korenchevsky, Dennison and Simpson 1935).

Somewhat similar facts may be true for progesterone, but so far this has not been observed to the same extent, possibly because the compound, as a diketone, may be less soluble in body fluids, and possibly because rabbits, the usual test animals for progesterone, seem to be more economical in their use of administered steroid sex hormones.

The rate of absorption of compounds given intraperitoneally or subcutaneously must depend largely on their solubility in water, and it might be expected, therefore, that the effectiveness of water-soluble hormones which are easily destroyed or excreted once they reach the circulation would be greatly affected by the method of administration. Actually there is good reason to suppose that this is so. The gonadotropic hormones of the anterior pituitary or of urine are more effective when a certain dose is divided into a large series of small injections; activity may be reduced by intramuscular injection, which favours rapid absorption (e.g. prolactin, Bates and Riddle 1936); on the other hand, addition of a protein precipitant such as zinc sulphate to the solution, which delays absorption, increases greatly the activity of pituitary gonadotropic extract (Maxwell

1934), as does the addition of merthiolate to thyrotropic extracts (Ch'en and van Dyke 1936).

Good examples of the same phenomenon are to be found among non-sex hormones. Thus the assay of extracts of the adrenal cortex, of which the active principle seems to be water-soluble, is complicated by the fact that multiple injections are necessary unless very large quantities are used (Bülbring 1937). It would seem that the esterification of corticosterone, or even of reasonably pure extracts, might make possible much more efficient administration. The recent history of insulin is also relevant. With highly purified or crystalline preparations, the maximum effect of a dose was reached very soon after injection and a technique of injection immediately before meals was adopted, so that the readily absorbed material would be available to deal with the products of digestion. When the active substance is combined with protamine, the routine needs some alteration; few injections are required, and administration must take place some hours before a meal. It seems, in fact, that research on many of the hormones concerned in producing continued effects passes through similar phases. A stage of extract purification, and ultimate isolation of the active substance, is followed by one in which the necessity is realized of adding extraneous materials to solutions of the highly purified substance, in order to render absorption sufficiently slow, and to make possible high effectiveness, without prohibitively frequent injections.

Analogous facts have been known for some time in the field of chemotherapy (see, for instance, Dale 1921) and have recently become important to immunologists (see, for instance, Hartley 1935). It is possible, therefore, that the knowledge obtained from recent intensive study of the optimum conditions of hormone administration may be of direct application in other fields, especially in view of de Fremery's* finding that antigonadotropic serum, fully effective against ordinary solutions of the antigen, may be ineffective when the absorption of the antigen in the test animal is delayed by the addition of zinc sulphate to the solution.

The present paper deals with a further study of rate of absorption of sex hormones, and in particular with the mechanism of the effect of esterification. When these experiments were begun it seemed certain that the effects of palmitic acid, increased volume of oil, etc., depended on decreasing the rate of absorption from the subcutaneous depot of the hormone, but it was not known whether the greater efficiency caused by esterification was due to slow hydrolysis of the ester in the circulation, combined with the body's inability to destroy or excrete it unhydrolysed. The experiments described below seem to show conclusively that the aliphatic esters are less soluble in body fluids than the free hormone and are more slowly and often more effectively absorbed.

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* Personal communication to the authors.

TECHNIQUE

The substance used, the nature and amount of solvents, method of injection, etc. are described in the text. All doses of esters are recorded in terms of free hormones, except in tablet administration.

Castrated rat test—Dissection and weighing of the accessory glands were all as previously described (Callow and Deanesly 1935). Castration, except in maintenance experiments, was carried out when the body weight was 40–60 g.; the animals were used at varying periods after a month from castration. Throughout this paper P. and S.V. are used respectively for prostate and seminal vesicles.

Capon comb test—Measurements were carried out as previously described (Callow and Parkes 1935). Duration and nature of treatment and time of making measurements were all as recorded in the text.

Capon plumage test—This test for oestrogenic activity, depending on the production of the female coloration on the normally black breast feathers of the Brown Leghorn capon, was carried out as described by Parkes (1937), who also discusses the relevant points of the physiology of feather growth.

Test on uterus of immature rabbit—Some tests were carried out by the method described by McPhail (1936).

Tablet technique—Tablets for implantation were made by compression of the undiluted crystalline hormone in a micro-pastille press. The animals were anaesthetised with ether and the tablets were implanted under the skin through a small slit which was afterwards closed by a stitch. When the animal was killed, adherent tissue was, as far as possible, removed from the tablet, which was then dried in a desiccator before reweighing. The weights of the residues given in Tables III, IV and V can, of course, only be regarded as approximate.

SUBDIVISION OF THE TOTAL DOSE

There is reason to suppose (McPhail 1934; Klein and Parkes 1937) that the wastage of administered hormones by the rabbit is much less than that by the rat. McPhail (1936) found that the maximum development possible in the uterus of the immature rabbit in 5 days could be induced by three doses of 5γ oestrone. In a special experiment on the effect of subdivision of the dose in immature rabbits, we have administered 12γ as six daily doses of 2γ, three doses of 4γ on alternate days, two doses of 6γ with an interval of 3 days and one dose of 12γ; in each case the animals were killed at the end of the sixth day and the total volume of oil given was kept constant. The results (Table I) show that the effect of subdivision of the total dose is nothing like so great as in the rat.

TABLE I—EFFECT OF VARIOUS DEGREES OF SUBDIVISION ON THE EFFECTIVENESS IN IMMATURE RABBITS OF A TOTAL DOSE OF 12γ OESTRONE

No. of doses over 6 days	Amount per dose γ	No. of rabbits	Breed	Average body weight g.	Average uterus weight mg.
6	2	6	Himalayan	528	605
3	4	6		540	609
2	6	5		624	597
1	12	3		787	529
6	2	4	Dutch	545	682
3	4	4		580	324
2	6	5		686	697
1	12	4		700	394

NATURE OF SOLVENT

Both aqueous and oil solutions of oestrone are made more effective by divided dosage, but the effect is more marked with the aqueous ones, suggesting that absorption is more rapid from these. The absorption of oestrone from castor oil seems to be slow (Deanesly and Parkes 1933), possibly because of the high solubility of the compound in this oil. Testosterone seems to be inactive in liquid paraffin; presumably the animal is unable to obtain the hormone from such an inert medium (Miescher, Wettstein and Tschopp 1936). Androsterone is more than three times as effective given in propylene glycol as in olive oil (Deanesly and Parkes 1936). Propylene glycol is miscible with water, and the androsterone is probably precipitated when the solvent is diluted with body fluids, giving the effect of administration of a crystal mush (see p. 286). The same explanation may apply to the increased activity of androsterone in alcoholic solution observed by Callow and Deanesly (1935).

Testosterone—A total of 2 mg. of free testosterone in 1·0 c.c. 70 % ethyl alcohol given over 10 days was just about as effective as when given in oil. The same dose in a total of 1·0 c.c. propylene glycol has a low effectiveness.

Oestradiol benzoate—The effect on the plumage of Brown Leghorn capons of 1 mg. oestradiol benzoate was considerably curtailed by administering it in 1 c.c. propylene glycol instead of in oil, the average duration of response in five birds being reduced from 9 days (Parkes 1937) to 5½ days.

ADDITION OF FATTY ACID

Miescher, Wettstein and Tschopp (1936) showed that the effectiveness of testosterone could be greatly increased by the addition of palmitic acid to the solution. This finding was confirmed and extended to androsterone by Deanesly and Parkes (1936) and to androstanedione by Parkes (1936). The same thing holds for *trans*-androstanediol, and Fig. 2 shows that palmitic acid restores the effectiveness of *cis*-androstanediol and *trans*-androstenediol lost by increasing the amount of oil. Addition of palmitic acid also greatly increases the effectiveness of testosterone propionate, if given as one injection only in 1 c.c. of oil. (P. + S.V., 67 mg. and 391 mg.)

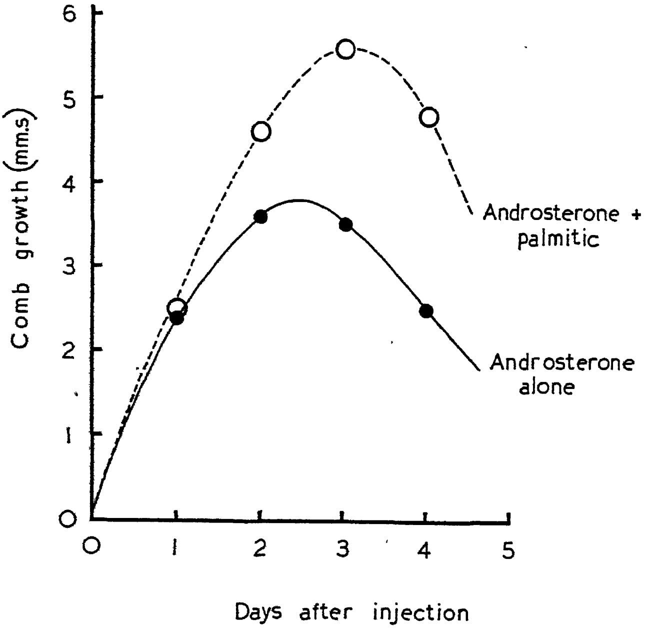

FIG. 1—Influence of palmitic acid on the effectiveness of a single dose of 1 mg. androsterone. Five capons in each group.

The effect on the capon comb of 1 mg. of androsterone given as a single injection is also enhanced, both in intensity and duration of action, by the addition of palmitic acid (fig. 1).

The duration of the effect on the plumage of Brown Leghorn capons of 1 mg. oestrone was about doubled by the addition of 0·5 g. of palmitic acid to the 4 c.c. oil solution.

ESTERIFICATION

Following the work of Miescher, Wettstein and Tschopp (1936) much has now been written about the active esters of testosterone and other male hormones. We have examined a number of inactive esters which are as important theoretically as the active ones. *cis*-Androstanediol 3-benzoate in total doses of 5 and 10 mg., given daily over 10 days in a total of 5 c.c. oil, evoked no appreciable response. Similarly a total of 10 mg. of *trans*-androstenediol 17-benzoate given in one injection or in ten injections over 10 days gave no response.

Two experiments were carried out on testosterone succinate dissolved in propylene glycol, but no appreciable activity was demonstrated.

Oestriol monoglucuronide—2 mg. of this compound injected intramuscularly in 1 c.c. propylene glycol failed to affect capon plumage.

Benzoylation will certainly decrease the solubility in body fluids of androstanediol and *trans*-androstenediol, whereas succinylation will certainly increase the solubility of testosterone. A possible explanation of the inactivity of these compounds is that the benzoates become too insoluble to absorb and the succinate too soluble to remain in the body long enough to produce any visible reaction.

INCREASE OF THE VOLUME OF MEDIUM

The effect of increasing the total amount of oil used to administer 10 mg. of androsterone or 5 mg. of androstanedione or 2 mg. testosterone has already been shown (Deanesly and Parkes 1936). The data given by Parkes (1936) also suggests that the effectiveness of 2 mg. of testosterone propionate given in a single injection 10 days before killing is enhanced when the volume of oil is increased from 2 to 5 c.c.

trans-*Androstanediol*—Increase of the volume of oil from a total of 1 c.c. to a total of 10 c.c. increased the effectiveness slightly. This compound is readily soluble in oil.

cis-*Androstanediol* and trans-*androstenediol*—Both these substances are only sparingly soluble in arachis oil; solutions more concentrated than 1 mg. per c.c. crystallize out in the course of a few hours at room temperature. 10 mg. of *trans*-androstenediol in 4 c.c. oil over 10 days caused fair growth (P. + S.V. = 186 mg.) of the accessory glands (Deanesly and Parkes 1936), a finding since confirmed for *trans-trans*-androstenediol (10 mg. in 2 c.c. oil gave P. + S.V. = 197 mg.), and it was a surprise, therefore, to find that the same amount given over 20 days in 10 c.c. of oil produced negligible growth. It seemed that increasing the volume of oil to a point where the solution was stable resulted in decreased effectiveness, and that the high effectiveness of the concentrated solution was due to delayed absorption of a solution likely to crystallize out shortly after injection. In a preliminary experiment with androstanediol, the effectiveness of 4·4 mg. was not altered by the increase of the total oil from 2 to 5 c.c. In a definitive experiment 10 mg. of androstanediol and 10 mg. *trans*-androstenediol were injected each in totals of 1, 2 and 5 c.c. of oil. In both cases the two more concentrated solutions became a mixture of oil and crystals after an hour or two. This was injected daily without reheating. The results, fig. 2, show definitely that the crystal mush is a more effective form of administering these two hormones than is an oil solution.

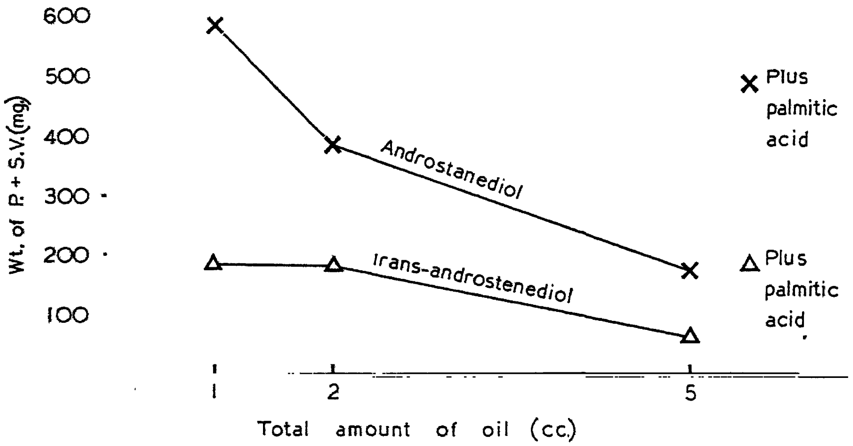

Fig. 2—Effect of volume of oily medium on effectiveness of *cis*-androstanediol and *trans*-androstenediol in castrated rats.

Testosterone propionate—It was found (Parkes 1936) that testosterone acetate given daily is not made more effective by increasing the volume of oil, but experiments with the propionate show that when the whole dose is given in one injection and the animals killed 10 days later, the result can be greatly influenced by the amount of oil used for the single injection. (5·0 c.c. oil, P.+S.V. = 275 mg.; 0·2 c.c. oil, P.+S.V. = 60 mg.)

A total of 2 mg. of the propionate in a total of 0·5 c.c. propylene glycol given daily over 10 days produced P.+S.V. = 483 mg. Increase of the propylene glycol to 1·0 c.c. decreased the response to 375 mg.

Oestradiol monobenzoate—Greenwood, Blyth and Callow (1935) were unable to show that the effectiveness of androsterone on the capon comb was influenced by the volume of oil used for the intramuscular injection. An analogous experiment showed that the power of oestradiol benzoate to feminise the plumage of Brown Leghorn capons was the same whether the volume of oil was 0·1 or 4·0 c.c.

IMPLANTATION OF CRYSTALS AND TABLETS

The results obtained with the crystal mushes of androstanediol and *trans*-androstenediol suggested that administration of hormone in solid form might be highly effective.

Testosterone—As a first experiment tablets of testosterone varying from 1·8 to 3·7 mg. were implanted subcutaneously through a small slit in the skin. The rats were killed 10 days later, and were found to have an average P.+S.V. weight of 588 mg., a response doubly as good as that given by twice daily injections of the same total amount of free hormone in a large amount of oil, better than that given by 2 mg. testosterone propionate administered as one injection in oil, and approaching that given by 2 mg. of the propionate administered in ten daily injections (see fig. 3). In the course of repeating this experiment it was observed that the tablets were not entirely absorbed in 10 days, and the residues were therefore recovered, cleaned of adherent tissue and dried and weighed, so that the actual amount absorbed by the rat could be determined. The results on four groups of rats receiving tablets of various sizes are shown in Table II. The responses to tablet administration are remarkably good, yet only 50–75% of the weight of the tablets of this size is used up in 10 days.

It will be seen (Table II) that although appreciably more testosterone was absorbed by the rats receiving larger tablets, there was not a proportionate difference in the size of the accessory glands. In other words, with tablets larger than about 1 mg., more was absorbed than could be efficiently utilized.

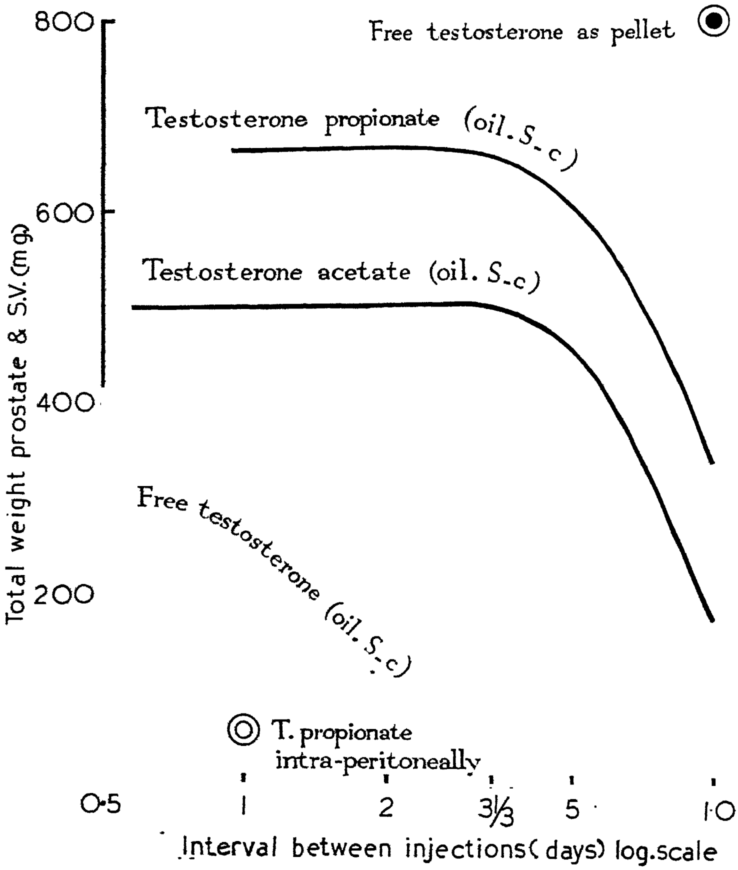

FIG. 3—Effectiveness of free testosterone in tablet form and of testosterone propionate given intraperitoneally in oil solution, compared with that of the free hormone, the acetate and the propionate according to degree of subdivision of the total dose.

TABLE II—EFFECTIVENESS OF TESTOSTERONE ADMINISTERED IN TABLET FORM

Average size of tablet mg.	Range of size mg.	Average amount absorbed mg.	No. of rats	Average P.+S.V. mg.
1·1	1·1–1·2	0·7	4	665
1·2	1·2–1·3	0·6	5	692
1·7	1·5–1·9	1·1	5	675
2·2	2·0–2·5	1·5	5	877

The experiment was continued by the reimplantation into a second set of rats of the tablet residues from the fifteen rats of the last three groups listed in Table II. No inactivation of the testosterone had taken place while lying in the subcutaneous tissue. Residues, totalling 8·3 mg., were obtained suitable for reimplantation. These were implanted into twelve rats killed 10 days later and produced an average P. + S.V. weight of 362 mg. Very small residues were again recovered from three of these rats, and reimplanted into three more producing an average P. + S.V. weight of 80 mg. after 10 days. The original 25·5 mg. of testosterone with which the fifteen rats were implanted had thus produced by the end a total P. + S.V. weight of 15·8 g. or 1 mg. testosterone produced 650 mg. P. + S.V., an astonishing result compared with those obtained by its administration in concentrated oil solution.

In a further maintenance experiment five adult rats were castrated and on the same day received tablets of testosterone averaging 1·4 mg. Fourteen days later about nine-tenths of the tablets had been absorbed: the accessory glands, far from showing any castration changes, were above the normal for the body weight of the animals. The prostates averaged 70% above normal and the seminal vesicles 90% above normal. It may be concluded therefore that under optimum conditions 50γ of testosterone daily would be enough for maintenance and normal growth of the accessories in adults of this size.

Testosterone propionate—A similar experiment was carried out with testosterone propionate in solid form, large aggregates of crystals being used instead of compressed tablets. In the first experiment in which the residues were not examined, it was found that crystals averaging 2·4 mg. produced an average P. + S.V. weight of 440 mg. or less than in the experiment with free testosterone. In the second experiment, crystals averaging 1·2 mg. were implanted for 10 days, the residues reimplanted for 10 days and their residues again reimplanted for 10 days (Table III), and found to be still active. By adding the weights of the accessory glands of the thirteen animals stimulated by the original 6 mg. of testosterone propionate, it was found that they totalled 3·914 g. The average growth per mg. of testosterone propionate* was 652 mg. or exactly the same as per mg. of free testosterone. Naturally in both these experiments some wastage took place in transferring the residues, and small traces were left at the end of the experiment, but the general agreement is striking. In a third experiment with testosterone propionate crystals, implanted for 21 days, from which the residues were implanted for a further 20 days (Table III), good growth was obtained but the total weight of accessory glands produced per mg. of propionate was lower than in the previous test—445 mg. This is probably due to the greater amounts of hormone required in a long duration experiment, purely for maintenance of the already enlarged glands. It seems, therefore, that whereas testosterone tablets are absorbed to the extent of $\frac{2}{3}$–$\frac{3}{4}$ in 10 days, the propionate tablets are only $\frac{1}{5}$–$\frac{1}{4}$ absorbed in 10 days, or up to $\frac{1}{2}$ in 20 days. From this slower absorption of the propionate it is clear that it is very much less soluble in body fluids than the free hormone, a significant fact in considering the mechanism of the increased effectiveness of the propionate given subcutaneously in oil solution.

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* Equivalent weight of free hormone not calculated.

TABLE III—EFFECTIVENESS OF TESTOSTERONE PROPIONATE ADMINISTERED IN TABLET FORM

No of experiment	Average size of tablet mg.	Source of tablets	Duration of experiment days	Average amount absorbed mg.	No. of rats	Average P.+S.V. mg.
1	2·4	New	10	Not determined	5	440
2 A	1·2	New	10	0·2	5	437
2 B	1·0	Residues from 2 A	10	0·3	5	248
2 C	1·1	Residues from 2 B	10	Not determined	3	163
3 A	2·7	New	21	0·9	5	803
3 B	1·8	Residues from 3 A	20	0·7	5	417

cis-*Androstanediol*—Tablets with an average weight of 5·9 mg. were implanted into five rats which were killed 10 days later. The average P.+S.V. weight was 242 mg. Even considering the dose as 5·9 mg., this response is better than that obtained from any other method of administration except the concentrated oil solutions. But residues averaging 4·7 mg. were recovered from these five rats, so that the effective dosage was little over 1 mg. A series of reimplantations for 10-day periods was then carried out; the residues decreased in size with each successive reimplantation, but they remained active. The results are listed in Table IV and may be summarized as follows:

a—With androstanediol tablets ranging between 3 and 6 mg., one-eighth to one-fifth is absorbed in 10 days, i.e. the substance appears to be slightly less soluble in body fluids than testosterone propionate.

b—After nine implantations, when the residues weighed 5·7 mg. as compared with the original total of 29·2 mg., the total weight of prostate and seminal vesicles produced was 6·65 g., or nearly 300 mg. per mg. of androstanediol. Given by this technique, therefore, androstanediol is more than ten times as effective as when given in dilute oil solution (see p. 286).

TABLE IV—EFFECTIVENESS OF ANDROSTANEDIOL TABLETS TRANSFERRED TO SUCCESSIVE GROUPS OF RATS

Experiment	Average amount implanted mg.	Source of tablets	No. of rats	Average residue mg.	Average amount absorbed mg.	Average weight P.+S.V. mg.
A	5·9	New	5	4·7	1·2	242
B	4·7	Residues from A	5	4·2	0·46	205
C	4·2	Residues from B	5	3·7	0·56	252
D	3·7	Residues from C	5	3·19	0·47	175
E	5·3	Residues from D	3	*	—	159
F	*	Residues from E	3	*	—	209
G	*	Residues from F	3	3·7	—	212
H	3·7	Residues from G	3	2·6	1·1	180
I	2·6	Residues from H	3	0·7	1·9	153

* Weights not obtained.
Total amount absorbed (or lost) after nine implantations = 23·5 mg.
Total P.+S.V. = 7·1 g.

In an experiment on the maintenance of the accessory glands of the castrated adult rat, five rats of average body weight 155 g. were each implanted on the day of castration with a tablet of androstanediol averaging in weight 4·7 mg. Fourteen days later, when the rats were killed, an average of 0·7 mg. androstanediol had been absorbed but the accessory glands were not fully maintained (P.+S.V. = 714 mg. compared with 864 mg. found in five normal rats of the same body weight). In a second test, five rats of average body weight 140 g. received tablets averaging 6·7 mg., of which an average of 1·8 mg. had been absorbed when the animals were killed 28 days later. The glands were fully maintained, P.+S.V. averaging 1291 mg. compared with 1149 mg. in five normal rats of the same final body weight. It seems, therefore, that the daily absorption under suitable conditions of 100γ androstanediol can maintain the accessory glands of the adult castrated rat. Reimplantation of the residues from these experiments gave expected results.

Other male hormone compounds—The following substances tested by tablet implantation were inactive or produced negligible growth over 10 days—androsterone, *trans*-androstenediol, *cis*-androstanediol-mono-3-benzoate, testosterone oxime and testosterone oxime propionate. These substances showed very little loss of weight and were presumably too insoluble to be active. Tablets of androsterone of about 5·7 mg., however, showed an average loss of 1 mg., but this proved insufficient, perhaps owing to rapid excretion, to produce much growth. The prostates showed a slight response, but the seminal vesicles were not stimulated.

The water-soluble compounds, testosterone succinate and androstanediol disuccinate, disappeared from the subcutaneous tissues in 7 days or less and gave negative results.

Oestrone—The prolonged effect of hormone administered in solid form can be beautifully demonstrated by the plumage response of Brown Leghorn capons. Four birds were used for an experiment in which crystals of oestrone, weighing between 2·0 and 3·2 mg., were implanted intramuscularly 11 days after the feathers had been stripped off the left side of the breast. The regenerating feathers were feminized from the time of implantation onwards. To examine the further effects, the right side of the breast was stripped 28 days after the implantation. In all four birds all the feathers growing after this stripping were completely female. Subsequently the left side and also the right side were again stripped. A summary of the experiment is given in Table V from which it will be seen that in three out of four birds female plumage continued to grow for nearly 3 months (see fig. 7 *b–d*). From the weights of the crystals implanted in these birds, it may be calculated that the absorption of about 30γ daily of free oestrone is adequate to feminize the plumage of Brown Leghorn capons. This result agrees remarkably well with that given by the most efficient ester in oil solution, oestradiol 3-benzoate 17-acetate, examined by Parkes (1937).

Table V—Effect of a single implantation (3.xi.36) of a crystal of oestrone on the plumage of Brown Leghorn capons

No. of bird	Weight of crystal implanted mg.	Condition of feathers regenerating after plucking on			
		Left 22.x.36	Right 2.xii.36	Left 31.xii.36	Right 2.ii.37
A 21	3·0	Female, except tip	All female	All female	Male
A 22	2·0	Female, except tip	”	”	”
A 23	3·2	Female, except tip	”	”	”
A 25	2·8	Female, except tip	”	Male	”

Route of administration

The usual technique is to make subcutaneous injections in rats and intramuscular injections in birds. In considering other possible routes of administration it is necessary to distinguish between a route which involves the substance entering the general circulation in order to reach the site of action, and those which depend on the local administration of the hormone to organs accessible from the exterior of the animal. The comb and feathers of the bird are, of course, among the best examples of externally accessible test objects. One might expect that local application would greatly reduce the amounts of active material required. Greenwood and Blyth (1935) were able to show that local injection of oestrone into the feather follicle, or of androsterone into the capon comb, increased the effectiveness of these substances. Fussgänger (1934) found that comb growth in the young chick could be caused by the application to the surface of the comb of remarkably small amounts of androsterone. In mammals, interesting results have been obtained by the intravaginal administration of oestrone solution (Berger 1935), by which means cornification may be obtained with very small amounts of oestrogen. De Fremery's work (1936) on unction of the goat udder is another example of the effectiveness of local application. Superficial application of oestrone to the skin may be effective even where the organ to be acted upon can only be reached via the circulation (Burrows 1934), but large doses are required.

Unction—Free testosterone, testosterone acetate and testosterone propionate have been administered by smearing the solution on the shaved back. A total of 1 mg. of testosterone in 0·5 c.c. oil given by daily unction for 10 days produced P. + S.V. of 78 mg., a definitely positive response. Twice the amount in propylene glycol gave little response. Testosterone acetate was not observed to be effective by this route, but 2 mg. of the propionate applied thus in propylene glycol solution over 10 days was also just active (P. + S.V. = 81 mg.).

These results show that for internal test objects unction is not a very efficient method of administration. For the comb and feathers of the capon, however, it has been very satisfactory. In the capon comb test for male hormone activity, carried out by five daily administrations, a total dose of 2·5γ by unction gave as large a response (6 mm.) as 500γ by injection, so that application by unction seems to be about 200 times more effective than the usual method. More remarkable is the prolonged effect obtained by unction of the free hormone. Fig. 4 shows the behaviour of the combs in two groups of capons. Both groups received 1 mg. testosterone in propylene glycol by a single direct unction to the comb, but one group received free testosterone and the other the propionate. Comb growth continues for only a very short time after injection of the free hormone, and the prolonged response following unction is probably due to slow absorption through the surface of the comb.

A somewhat similar result was obtained by unction of the feather tract with oestradiol and oestradiol benzoate just before the regenerating feathers appeared through the skin. 250γ free oestradiol, smeared over the whole of one breast tract, produced the type of response shown in fig. 5*d*. This is due to a stimulus of minimum effective intensity acting for a fairly long period, the exact converse to the response to 250γ oestradiol given by a single injection, which is of full intensity but of very short duration (fig. 5*a*) (see Parkes 1937, for discussion of the relevant details of the physiology of feather growth). The application by unction of 250γ oestradiol as benzoate leads to a very similar response, the stimulus being on the average little more prolonged and no more intense, so that the compound must be considered as less effective by unction. From these comb and plumage tests it is clear that the inferiority of the free hormone in duration of action is largely abolished when administration is by unction.

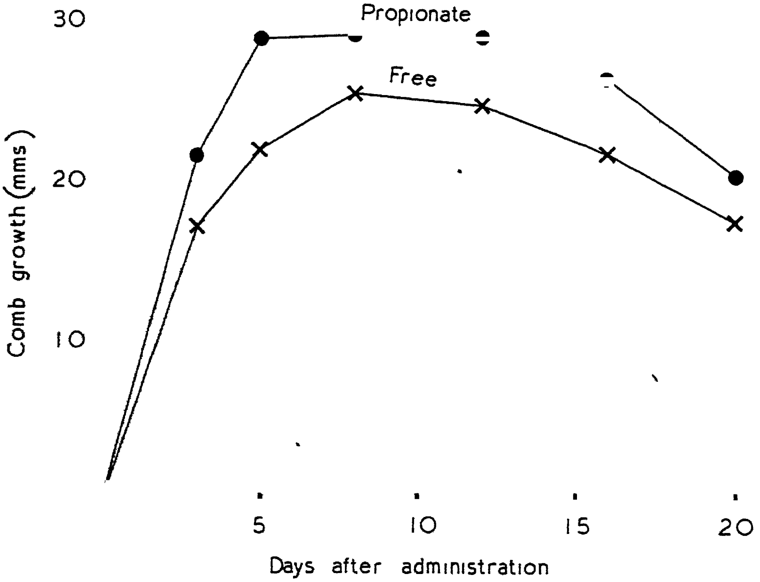


FIG. 4—Comparative effect of 1 mg. of testosterone and 1 mg. of testosterone propionate by direct unction to the comb. Groups of five capons.

Oral administration—Administration daily to castrated rats for 10 days in 0·1 c.c. propylene glycol of a total of 2 mg. testosterone free or as succinate or of 5 mg. androstanediol disuccinate failed to produce any significant response.

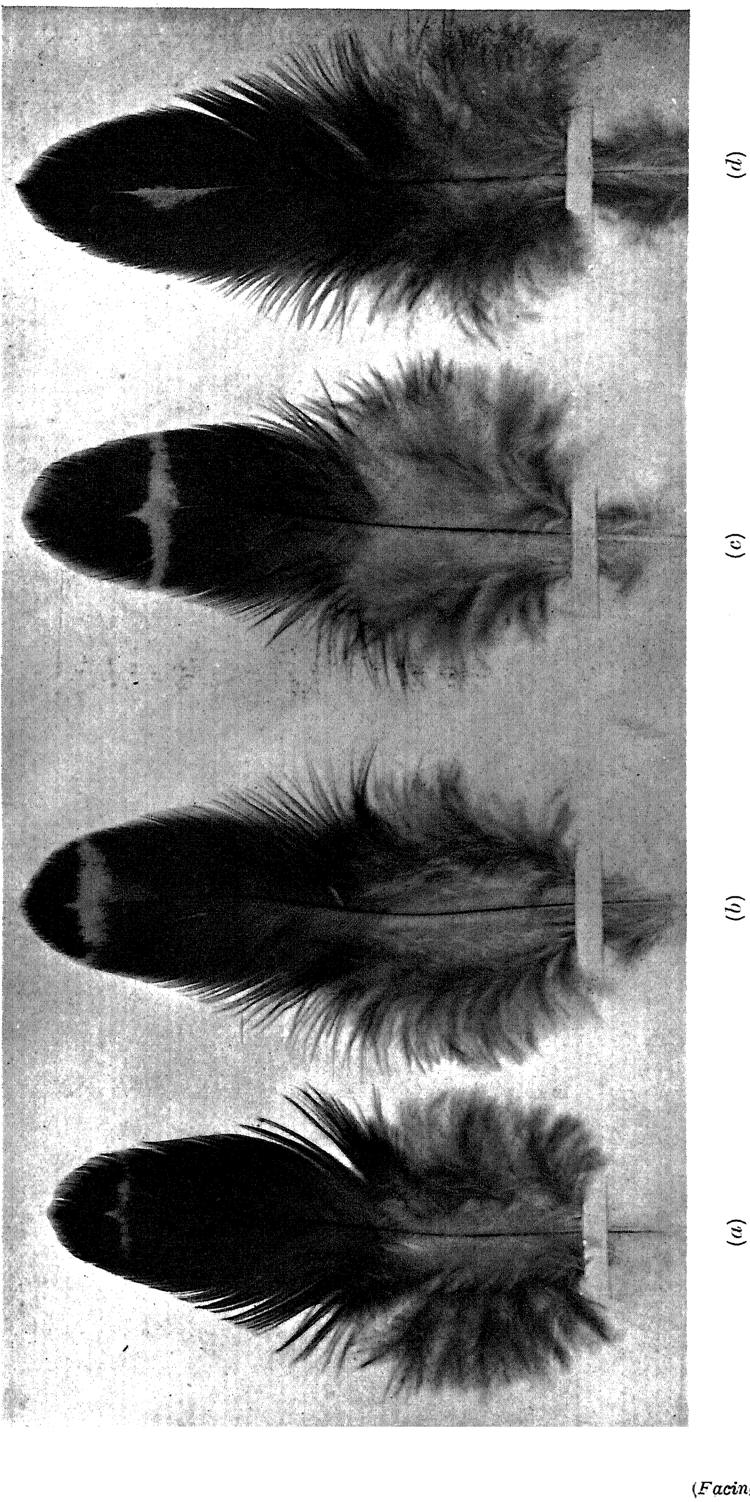
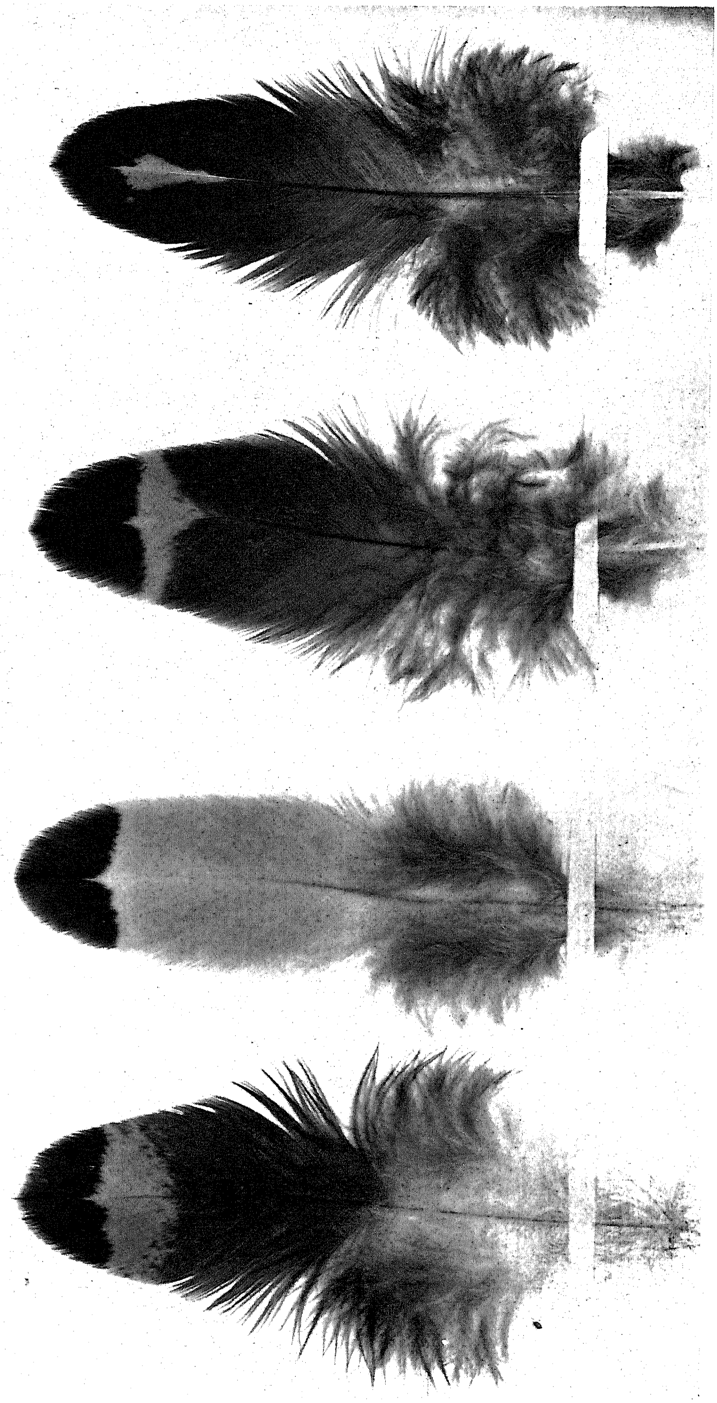


FIG. 5



(*a*) (*b*) (*c*) (*d*)

Fig. 6

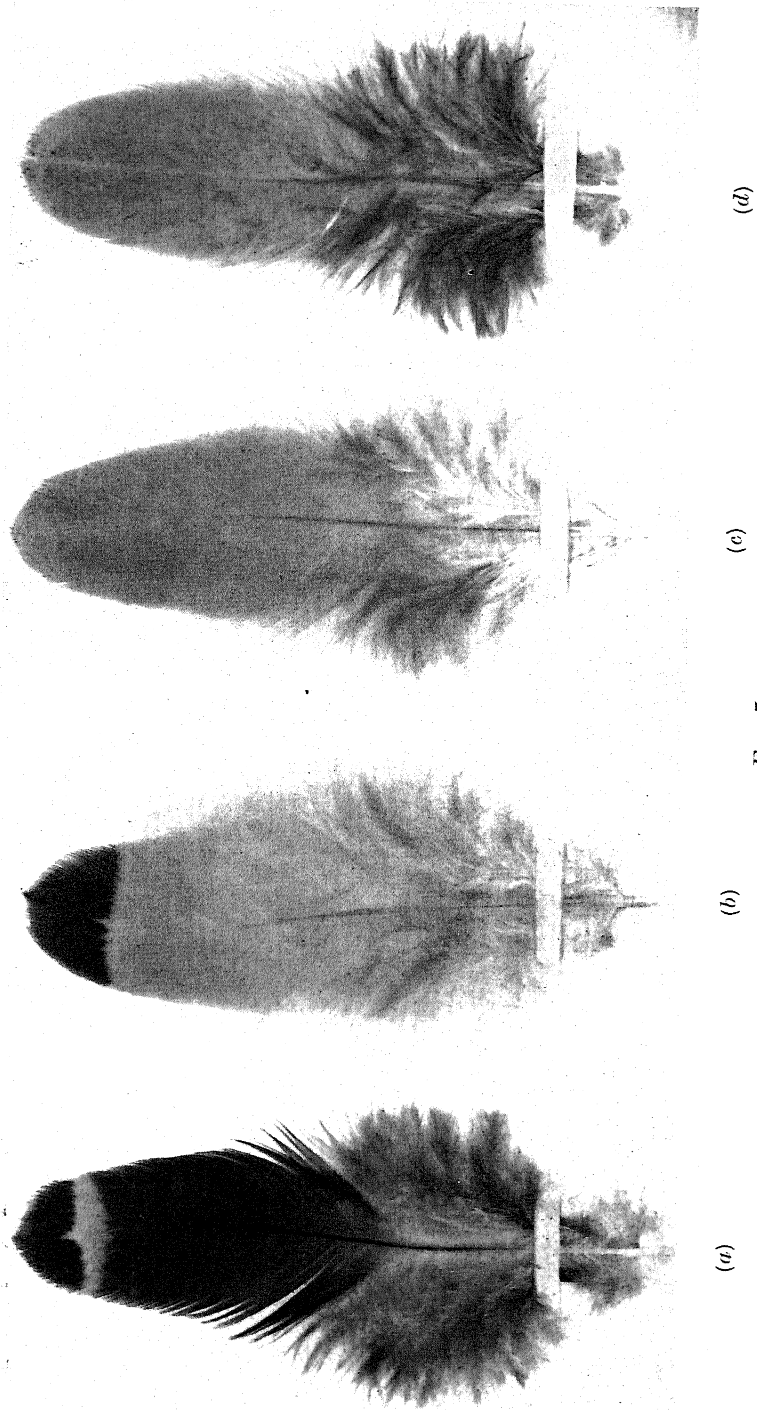
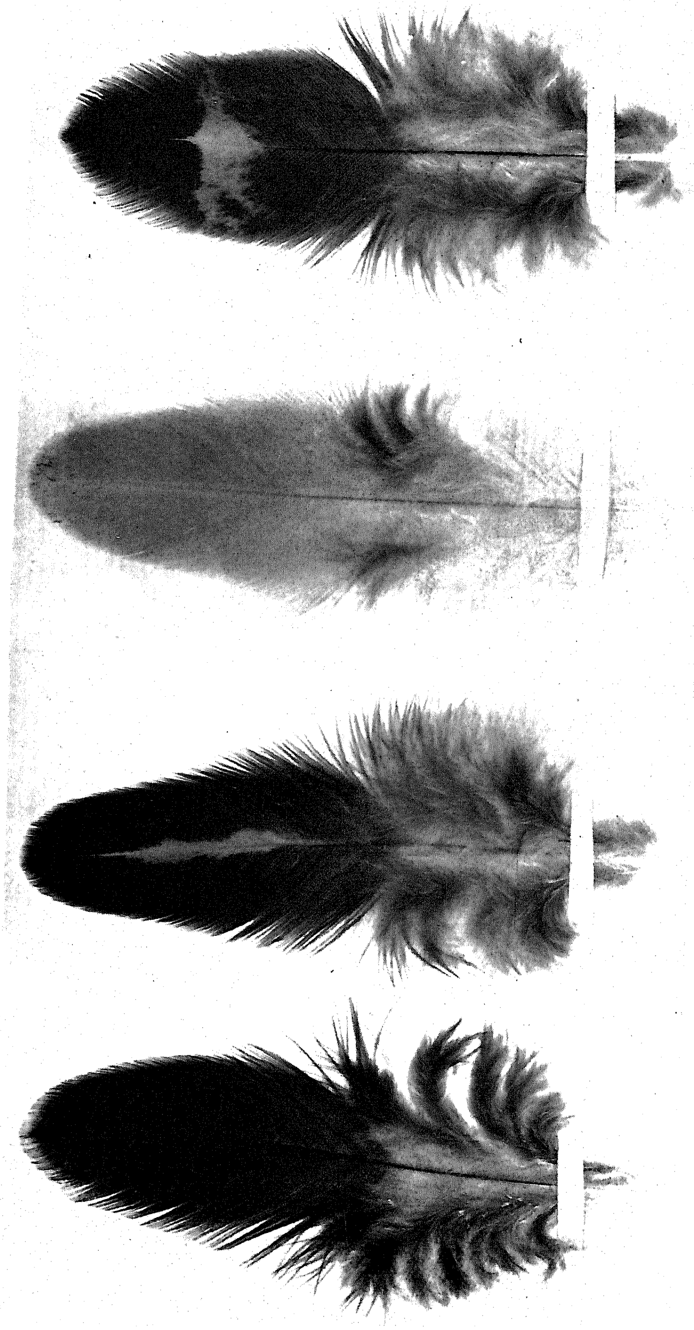


FIG. 7



(*a*) (*b*) (*c*) (*d*)

Fig. 8

Intraperitoneal injection—Experiments on the effectiveness of intraperitoneal injection were carried out in connexion with the study of the influence of the rate of absorption. Since the peritoneum is one of the best absorbing surfaces in the body, the results were instructive. Castrated rats were injected daily for 10 days with a total of 2 mg. testosterone, free or as propionate, in a total of 1·0 c.c. propylene glycol. The free hormone produced no demonstrable response, the propionate caused a P. + S.V. average of 51 mg. In other words intraperitoneal injection, even of the propionate, leads to such rapid absorption that effectiveness is minimal (see fig. 3).

Intravenous injection—As a sequel to the experiments on intraperitoneal injection, intravenous injections on rats would have been of interest, but continued administration by this route proved to be difficult. In one experiment, two intravenous injections at 5-day intervals of 1 mg. testosterone propionate in 0·25 c.c. propylene glycol were given. The treatment caused no response, but the fact is of doubtful significance, since two doses of the same amount in the same medium, given subcutaneously, also produced no response. Capons, however, offered excellent opportunities of investigating the efficiency of intravenous injection. 1 mg. of testosterone, free, or as propionate, was given by the ordinary intramuscular route and also by intravenous injection. In each case the total dose was given as one injection in 0·1 c.c. propylene glycol. The results showed that by intramuscular injection the propionate is about twice as good as the free hormone. The responses to the intravenous dosage were negligible, so that the effect of intravenous injection on the effectiveness of the ester was not determined. The experiment again shows the low effectiveness of testosterone when absorption is nearly or quite instantaneous.

The plumage experiments were much more satisfactory. Oestradiol benzoate (1 and 5 mg.), oestradiol dibenzoate (2·5 and 5·0 mg.), free oestradiol (5 mg.), and oestrone benzoate (5 mg.) were given intravenously in 1 c.c. propylene glycol. The results were quite clear-cut. The smaller dose of oestradiol benzoate produced a faint response of the rachis spot type. The larger dose resulted in a complete band of female colour across the feathers (fig. 6*c*), averaging about 6 mm. wide at the rachis, and similar to that produced by the intramuscular injection of rather more than 100γ. Seven capons injected intravenously with 5 mg. free oestradiol showed a more variable response, but in four the effect was equal to that given by the ester (fig. 5*c*). In other words, the free hormone is as effective when given intravenously as when given intramuscularly, while the monobenzoate is far less so. This presumably means that the prolonged action of the ester following intramuscular injection is due to delay of absorption from the site of injection rather than to delayed hydrolysis or non-excretability of the ester.

The results with the oestradiol dibenzoate and the oestrone benzoate were somewhat different. These substances are much less soluble than the others and difficulty was experienced in dissolving 5 mg. in a tolerated amount of propylene glycol. As a result, the material was partly out of solution when injected. The response to the injection of oestrone benzoate lasted about 10 days. This result may be due to the greater insolubility of the compound or to the fact that much of the dose was administered as a crystal mush. The oestradiol dibenzoate results were interesting. By intramuscular injection not less than 10 mg. is required to cause an effect, which is then prolonged (fig. 8*a*-*c*). By intravenous injection, however, 2·5 mg. gave a good though variable response (fig. 8*d*). This result shows that another effect of esterification—raising of the minimum effective dose—also depends on changes in absorbability from the site of injection.

Our best thanks are again due to Dr. R. K. Callow for much advice and assistance.

We are most grateful to Major G. W. Dunkin, Superintendent of the Institute's Farm Laboratories, for his help in organizing the poultry stock which has made possible the plumage and comb work recorded in this paper; to Miss E. A. H. Salmon we are much indebted for her constant care of the birds.

The male hormone compounds used in the work described were generously provided by Dr. K. Miescher and Messrs. Ciba.

SUMMARY

1—Further work has been carried out on the relation between method of administration and effectiveness of hormone preparations.

2—The response of the capon's comb and of the accessory glands of the castrated rat have been used in the study of androgenic activity and the response of the plumage of the Brown Leghorn capon in that of gynaecogenic activity.

3—Experiments are described on the effect of subdivision of the total dose, nature of the solvent, addition of fatty acid to the solution, esterification of the hormone, volume of medium and route of administration.

4—The influence of these different variables may be quite different for different substances, and it is concluded that no valid quantitative comparison of two compounds can be made without a careful examination of the conditions necessary for maximum effectiveness.

5—Certain of the free hormones show greatly increased effectiveness when given in dry form by the subcutaneous implantation of tablets or crystals. Recovery of the residue of the tablet from the rat after a 10-day period of experiment has made it possible to estimate the amount of hormone actually absorbed, and successive implantations of the same tablets to successive groups of rats has enabled the maximum effectiveness of a given dose to be determined.

6—The esters lose their increased effectiveness when given by intraperitoneal or intravenous injection and it is concluded that the prolongation of action by esterification is due to delay of absorption from the site of injection rather than to slow hydrolysis of the compound after absorption.

7—In general, it seems that the free hormones lose their inferiority to the esters if the method of administration makes absorption inevitably slow, whereas the esters lose their superiority to the free hormones if absorption is rapid or instantaneous.

REFERENCES

Bates, R. W. and Riddle, O. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **34**, 847–9.
Berger, M. 1935 *Klin. Wschr.* **14**, 1601.
Bülbring, E. 1937 *J. Physiol.* **89**, 64–80.
Burrows, H. 1934 *Brit. J. Surg.* **21**, 507–12.
Callow, R. K. and Deanesly, R. 1935 *Biochem. J.* **29**, 1424–45.
Callow, R. K. and Parkes, A. S. 1935 *Biochem. J.* **29**, 1414–23.
Ch'en, G. and van Dyke, H. B. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **35**, 491–2.
Dale, H. H. 1921 *Proc. Roy. Soc. Med.* **14**, 11.
David, K., Dingemanse, E., Freud, J. and Laqueur, E. 1935 *Hoppe-Seyl. Z.* **233**, 281–2.
Deanesly, R. and Parkes, A. S. 1933 *J. Physiol.* **78**, 155–60.
— — 1936 *Lancet*, **1**, 837–9.
de Fremery, P. 1936 *J. Physiol.* **87**, 50–51*P*.
Fussgänger, R. 1934 *Medizin. chem. Z.* **2**, 194–204.
Greenwood, A., Blyth, J. S. 1935 *Quart. J. Exp. Physiol.* **25**, 267–77.
Greenwood, A., Blyth, J. S. and Callow, R. K. 1935 *Biochem. J.* **29**, 1400–13.
Hartley, P. 1935 *Brit. J. Exp. Path.* **16**, 468.
Klein, M. and Parkes, A. S. 1937 *Proc. Roy. Soc.* B, **121**, 574–9.
Korenchevsky, V., Dennison, M. and Simpson, S. L. 1935 *Biochem. J.* **29**, 2131–42.
McPhail, M. K. 1934 *J. Physiol.* **83**, 145–56.
— 1936 *J. Pharmacol.* **9**, 672–8.

Maxwell, L. C. 1934 *Amer. J. Physiol.* **110**, 458–63.
Miescher, K., Wettstein, A. and Tschopp, E. 1936 *Schweiz. med. Wschr.* **66**, 310–12.
— — — 1936 *Biochem. J.* **30**, 1977–89.
Parkes, A. S. 1936 *Lancet*, **2**, 674–6.
— 1937 *Biochem. J.* **31**, 578–85.

DESCRIPTION OF PLATES 10–13

All feathers from the crop region of Brown Leghorn capons. Previous feathers plucked 10–15 days before administration of hormone except in fig. 7 *b-d* and fig. 8 *c*. Growth rate of feathers approximately 2 mm. per day.

FIG. 5—*a*, effect of 250γ free oestradiol intramuscularly in 4 c.c. arachis oil. *b*, effect of 4 mg. free oestradiol intramuscularly in 4 c.c. arachis oil. *c*, effect of 5 mg. free oestradiol, intravenously in 1 c.c. propylene glycol. The duration of effect is similar to that produced by subcutaneous injection of a similar amount. *d*, effect of 250γ free oestradiol, uncted to the whole of one breast feather tract in 1 c.c. arachis oil.

FIG. 6—*a*, effect of 250γ oestradiol-3-benzoate, intramuscularly in 4 c.c. arachis oil. *b*, effect of 4 mg. oestradiol-3-benzoate, intramuscularly in 4 c.c. arachis oil. *c*, effect of 5 mg. oestradiol-3-benzoate, intravenously in 1 c.c. propylene glycol. *d*, effect of 250γ oestradiol-3-benzoate, uncted to the whole of one breast feather tract in 1 c.c. arachis oil.

FIG. 7—*a*, effect of 4 mg. free oestrone intramuscularly in 4 c.c. arachis oil. *b–d*, effect of implantation of solid oestrone. Feathers from capon A 22, 3 mg. crystal of oestrone implanted intramuscularly on 3.xi.36. Left breast plucked 22.x.36, right breast 2.xii.36, left breast again on 31.xii.36 and right breast again on 2.ii.37. *b*, feather growing after plucking on 22.x.36. *c*, feather growing after plucking on 2.xii.36. *d*, feather growing after plucking on 31.xii.36.

FIG. 8—*a*, lack of effect of 4 mg. oestradiol dibenzoate, intramuscularly in 4 c.c. arachis oil. *b*, effect of 10 mg. oestradiol dibenzoate intramuscularly in 4 c.c. arachis oil. Prolonged stimulus of low intensity. *c*, effect of 20 mg. oestradiol dibenzoate intramuscularly in 10 c.c. arachis oil. Prolonged and intense stimulus. This feather began to grow immediately after the injection. *d*, effect of 2·5 mg. oestradiol dibenzoate, intravenously in 1 c.c. propylene glycol. Much of the hormone was out of solution at time of injection.

541.8:546.72

Some Conditions Governing the Solubility of Iron

By L. H. N. Cooper, Ph.D., F.I.C.

Assistant Chemist at the Plymouth Laboratory of the Marine Biological Association

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The amount of ferric iron in true solution in sea water has been found to be very small (Cooper 1935). In the course of an investigation on the iron supply of diatoms, H. W. Harvey observed that ferric hydroxide, even in alkaline solution, continuously gave off ferrous ions which reacted with 2 : 2′-dipyridyl to give the red tris-dipyridyl-ferrous ion. This led to the inquiry—how much iron can exist in true solution in sea water after equilibrium has been attained, and what are the conditions governing the equilibrium between ferrous and ferric ions?

With this end in view the theoretical calculations of 1935 have been extended and supplemented by a study of the oxidation-reduction potential of sea water (Cooper 1937). It was realized that such information would apply to the absorption of iron from alkaline solutions by other organisms.

FERROUS ION AS GOVERNED BY THE SOLUBILITY OF FERROUS HYDROXIDE

For the molar solubility of ferrous hydroxide the value $1 \cdot 8 \times 10^{-14}$ was derived from published data (Cooper 1935, Table VII). Kriukov and Awsejewitsch (1933) have further studied the solubility of ferrous hydroxide between *p*H 7·6 and 9, the region in which we are interested. They titrated ferrous sulphate with sodium hydroxide by Britton's method (1925*a*), and at *p*H 8·17, representative of sea water, found the product to be $2 \cdot 3 \times 10^{-14}$, in good agreement with the figure given above. This corresponds to 36 mg.-atom ferrous ion per litre.

On a rather uncertain basis Whitman, Russell and Davis (1925) suggested that the solubility of undissociated ferrous hydroxide in pure water is 0·047 mg.-atom Fe per litre. This quantity should be independent of *p*H and is too small to affect Kriukov and Awsejewitsch's results.

For our purpose the order of magnitude of this solubility product is all that is required. It is never likely to be of importance in sea water, since the concentration or activity of ferrous ion will usually be controlled by oxidation-reduction potential.

FERRIC ION AS GOVERNED BY THE SOLUBILITY OF FERRIC HYDROXIDE: TITRATION METHOD

Similarly Kriukov and Awsejewitsch determined the solubility product of ferric hydroxide to be about $0{\cdot}7 \times 10^{-36}$. Activity theory was not used, but in the following treatment this theory is introduced, since only with its aid is a true constant likely to be found.

Recently Bray and Hershey (1934) have drawn attention to the existence in aqueous solution of the ion, $FeOH^{++}$, and have calculated the thermodynamic equilibrium constant, K_1°, corresponding to equation (1):

$$Fe^{+++} + H_2O \rightleftharpoons FeOH^{++} + H^+, \tag{1}$$

$$K_1^\circ = \frac{a_{FeOH^{++}} a_{H^+}}{a_{Fe^{+++}}} = 6{\cdot}0 \times 10^{-3} \text{ at } 25^\circ \text{ C.} \tag{2}$$

This work was based mostly on a study of ferric perchlorate solutions (see p. 306). They have also calculated the molal, or concentration, equilibrium constant:

$$K_1' = \frac{m_{FeOH^{++}} m_{H^+}}{m_{Fe^{+++}}}, \tag{3}$$

where K_1' is a function of the ionic strength.

The total concentration of ionic ferric iron at a given ionic strength will be:

$$\begin{aligned} m_{\Sigma Fe_{ionic}} &= m_{Fe^{+++}} + m_{FeOH^{++}} \\ &= \frac{m_{H^+} + K_1'}{m_{H^+}} m_{Fe^{+++}}. \end{aligned} \tag{4}$$

Values of K_1' were given for a range of ionic strengths, μ up to $\mu^{\frac{1}{2}} = 1$ at 25° C. and up to $\mu^{\frac{1}{2}} = 0{\cdot}3$ at 35° C. The temperature coefficient of K_1' has been found from the relation: $\frac{d \log_{10} K_1'}{dt} =$ constant. It appears justifiable to extrapolate into the range, $\mu^{\frac{1}{2}} = 0{\cdot}3 - 0{\cdot}6$ at 35° C., in order to calculate K_1' at 18° C. for the temperature and concentrations of Kriukov and Awsejewitsch's experiments. Their data admit of the calculation of the ionic strengths of their solutions, so that by applying the appropriate values of K_1' the concentrations $m_{Fe^{+++}}$ and $m_{FeOH^{++}}$ may be computed by equation (4) (Table I).

The hydroxyl-ion activities are easily found from the observed $p\mathrm{H}$ values (strictly pa_{H}), taking pK_w as 14·23 at 18° C. We wish now to find the thermodynamic solubility products involving only activities. Unfortunately there are no data on the activity coefficients, $\gamma_{\mathrm{Fe}^{+++}}$ and $\gamma_{\mathrm{FeOH}^{++}}$, in solutions such as these, so that we must write

$$S_2 = a_{\mathrm{FeOH}^{++}} a^2_{\mathrm{OH}^-} = \gamma_{\mathrm{FeOH}^{++}} m_{\mathrm{FeOH}^{++}} a^2_{\mathrm{OH}^-},$$
$$S_3 = a_{\mathrm{Fe}^{+++}} a^3_{\mathrm{OH}^-} = \gamma_{\mathrm{Fe}^{+++}} m_{\mathrm{Fe}^{+++}} a^3_{\mathrm{OH}^-}.$$

The activity coefficient, $\gamma_{\mathrm{Fe}^{+++}}$, is not likely to exceed $\gamma_{\mathrm{La}^{+++}}$, the coefficient of lanthanum ion, nor is $\gamma_{\mathrm{Fe}^{++}}$ likely to exceed $\gamma_{\mathrm{M}^{++}}$, the coefficient of any bivalent ion such as Mg^{++}. We may therefore put

$$\gamma_{\mathrm{Fe}^{+++}} = < 0{\cdot}2 \quad \text{and} \quad \gamma_{\mathrm{FeOH}^{++}} = < 0{\cdot}3.$$

The results in Table I then give us

$$\frac{S_2}{\gamma_{\mathrm{FeOH}^{++}}} = 10^{-25\cdot 2}; \quad S_2 = < 10^{-25\cdot 7};$$
$$\frac{S_3}{\gamma_{\mathrm{Fe}^{+++}}} = 10^{-36\cdot 5}; \quad S_3 = < 10^{-37\cdot 2}.$$

TABLE I—SOLUBILITY PRODUCTS IN TERMS OF ACTIVITIES RECALCULATED FROM THE DATA OF KRIUKOV AND AWSEJEWITSCH (1933)

$p\mathrm{H}$	$\mu^{\frac{1}{2}}$	$K'_1 \times 10^3$ at 18° C.	$m_{\mathrm{Fe}^{+++}} \times 10^3$	$m_{\mathrm{FeOH}^{++}} \times 10^3$	$\frac{S_2}{\gamma_{\mathrm{FeOH}^{++}}} = m_{\mathrm{FeOH}^{++}} a^2_{\mathrm{OH}^-}$	$\frac{S_3}{\gamma_{\mathrm{Fe}^{+++}}} = m_{\mathrm{Fe}^{+++}} a^3_{\mathrm{OH}^-}$
3·65*	0·367	1·362	0·265	1·61	$10^{-23\cdot 97}$	$10^{-35\cdot 32}$
2·98	0·386	1·350	1·86	2·41	$10^{-25\cdot 12}$	$10^{-36\cdot 48}$
2·85	0·416	1·308	4·57	4·04	$10^{-25\cdot 15}$	$10^{-36\cdot 48}$
2·68	0·517	1·215	15·5	8·9	$10^{-25\cdot 15}$	$10^{-36\cdot 46}$
2·32	0·689	1·144	37·2	16·6	$10^{-25\cdot 60}$	$10^{-37\cdot 16}$
Accepted values					$10^{-25\cdot 2}$	$10^{-36\cdot 5}$

* Examination of the data shows that the results at $p\mathrm{H}$ 3·65 are likely to be less accurate than the others and have therefore been neglected in arriving at the accepted values.

FERRIC ION AS GOVERNED BY THE SOLUBILITY OF FERRIC HYDROXIDE: POTENTIAL MEASUREMENTS

The potential, E^h, of a system containing ferrous and ferric ions at activities $a_{\mathrm{Fe}^{++}}$ and $a_{\mathrm{Fe}^{+++}}$, will be given by

$$\mathrm{E}^h = \mathrm{E}^\circ + \frac{RT}{F} \ln \frac{a_{\mathrm{Fe}^{+++}}}{a_{\mathrm{Fe}^{++}}}, \tag{5}$$

where E° is the standard potential of the system when the activities of the two ions are equal. When the system is saturated with ferric hydroxide in equilibrium with the solid phase, the activity of the ferric ion, $a_{Fe^{+++}}$, is controlled by the hydroxyl ion and consequently by the hydrogen ion activity. Thus

$$a_{Fe^{+++}} = \frac{S_3}{a^3_{OH^-}} = \frac{S_3 a^3_{H^+}}{K^3_w}, \tag{6}$$

$$\therefore \quad E^h = E^\circ + \frac{RT}{F} \ln \frac{S_3 a^3_{H^+}}{K^3_w a_{Fe^{++}}}.$$

Replacing $a_{Fe^{++}}$ by $\gamma_{Fe^{++}} m_{Fe^{++}}$ and converting to common logarithms, we get at 18° C.

$$E^h = E^\circ + 0{\cdot}058 \log \frac{S_3 a^3_{H^+}}{K^3_w \gamma_{Fe^{++}} m_{Fe^{++}}}. \tag{7}$$

Let $$E^\circ_I = E^\circ + 0{\cdot}058 \log \frac{S_3}{K^3_w \gamma_{Fe^{++}}}. \tag{8}$$

Then $$E^h = E^\circ_I - 0{\cdot}058 \log m_{Fe^{++}} - 0{\cdot}174\, pH. \tag{9}$$

To be valid this expression requires only that ferric ion shall be in equilibrium with ferric hydroxide. Presence of other ions such as $FeOH^{++}$ or $FeCl_6^{---}$ will have no effect. Kriukov and Awsejewitsch derived expressions similar in form to (8) and (9) but using concentrations instead of activities and containing no activity coefficient, so that instead of equation (8) they had

$$E^\circ_I = E^\circ + 0{\cdot}058 \log \frac{S_3}{K^3_w}. \tag{10}$$

Their measurements were made between pH 3·5 and 6, more nearly approaching the pH of sea water than most work on the subject. Variations in $\gamma_{Fe^{++}}$ with dilution and with the ionic strengths of the solutions are to be anticipated and the Russian authors results for E°_I show just such small variations from the mean value, 1·011 V as would be expected. For the present we shall assume that E°_I and $\gamma_{Fe^{++}}$ can be treated as approximately constant and will rearrange equation (8) to give

$$\log \frac{S_3}{\gamma_{Fe^{++}}} = \frac{E^\circ_I - E^\circ}{0{\cdot}058} - 3pK_w. \tag{11}$$

From other measurements of their own, they took the standard potential of the ferric-ferrous electrode as 0·667 V, a value much lower than that generally accepted, viz. 0·747 V. Bray and Hershey (1934), after allowing for the presence of $FeOH^{++}$, consider that this latter value should be raised still further to 0·772 V. For our purpose this has much to recommend it, so that from equation (11)

$$\log \frac{S_3}{\gamma_{\mathrm{Fe}^{++}}} = \frac{1{\cdot}011 - 0{\cdot}772}{0{\cdot}058} - 3 \times 14{\cdot}23$$
$$= 10^{-38{\cdot}57}.$$

If the rather uncertain activity coefficient of ferrous ion is taken as 0·2 (see above), the solubility product S_3 becomes $10^{-39{\cdot}2}$.

Kriukov and Awsejewitsch found departures from theory above *p*H 6 which they attributed to a number of factors affecting their experiments. None of these is likely to arise in a study of sea water up to *p*H 8·5. Reviewing all the data for the thermodynamic solubility product of ferric hydroxide (Table II), the best value is considered to be

$$S_3 = a_{\mathrm{Fe}^{+++}} a^3_{\mathrm{OH}^-} = 10^{-38{\cdot}6}.$$

TABLE II—SOLUBILITY PRODUCT OF FERRIC HYDROXIDE AT 18° C.

Author	Method	$S_3 \times 10^{38}$	$\log_{10} S_3$
Ruff and Hirsch (1925)	Fractional precipitation	110	−35·96
Kriukov and Awsejewitsch (1933)	Titration	70	−36·15
,, ,,	Electrometric	32	−36·5
,, ,, (revised)	Titration	6·3	−37·2
,, ,, (revised)	Electrometric	0·27	−38·6
,, ,, (revised)	Electrometric and putting $\gamma_{\mathrm{Fe}^{++}} = 0{\cdot}2$	0·05	−39·2
Britton (1925*b*)	Electrometric	2	−37·7
Jellinek and Gordon (1924)	Electrometric titration of tartrate	1	−38·0

Using this measurement and the standard free energies of ferric and hydroxyl ions given in "International Critical Tables", 7, the standard free energy of formation of ferric hydroxide has been found to be about −168,000 cal.

From Bray and Hershey's figures for K_1° at 25 and 35° C., K_1° at 18° C. has been calculated to be $3{\cdot}7 \times 10^{-3}$, and may be combined with the above value for S_3 to give the thermodynamic solubility product

$$S_2 = a_{\mathrm{FeOH}^{++}} a^2_{\mathrm{OH}^-}.$$

Since
$$K_1^\circ = \frac{a_{\mathrm{FeOH}^{++}} a_{\mathrm{H}^+}}{a_{\mathrm{Fe}^{+++}}} = \frac{a_{\mathrm{FeOH}^{++}} a^3_{\mathrm{OH}^-} a_{\mathrm{H}^+}}{a_{\mathrm{Fe}^{+++}} a^3_{\mathrm{OH}^-}} = \frac{S_2 K_w}{S_3},$$

$$\therefore \quad S_2 = \frac{K_1^\circ S_3}{K_w} = \frac{3{\cdot}7 \times 10^{-3} \times 10^{-38{\cdot}6}}{10^{-14{\cdot}23}} = 10^{-26{\cdot}8}. \qquad (12)$$

This compares with $10^{-25\cdot 2}$ by the titration method and is probably more nearly correct.

Using the values $S_2 = 10^{-26\cdot 8}$ and $S_3 = 10^{-38\cdot 6}$, the maximum activities of the $FeOH^{++}$ and Fe^{+++} ions able to exist in equilibrium in sea water at 18° C. have been calculated (Table III).

TABLE III—ACTIVITY OF Fe^{+++} AND $FeOH^{++}$ IN SATURATED SOLUTIONS OF FERRIC IRON AT 18° C.; $pK_w = 14\cdot 23$

pH	$a_{Fe^{+++}}$ mg.-atom/m.³	$a_{FeOH^{++}}$ mg.-atom/m.³
8·5	$10^{-15\cdot 4}$	$10^{-9\cdot 3}$
8·0	$10^{-13\cdot 9}$	$10^{-8\cdot 3}$
7·5	$10^{-12\cdot 4}$	$10^{-7\cdot 3}$
6·0	$10^{-7\cdot 9}$	$10^{-4\cdot 3}$
4·0	0·013	0·5

THE RELATION BETWEEN THE ACTIVITY OF FERROUS ION AND OXIDATION-REDUCTION POTENTIAL

Let us consider the equilibrium

$$Fe^{+++} + \epsilon \rightleftharpoons Fe^{++}$$

in a solution saturated with ferric ion but not with ferrous ion. Equation (9) then applies, and if we insert $E_1^\circ = 1\cdot 011$ V and neglect the activity coefficient, $\gamma_{Fe^{++}}$, at high dilutions, we get

$$E^h = 1\cdot 011 - 0\cdot 058 \log a_{Fe^{++}} - 0\cdot 174\, pH. \tag{13}$$

When discussing the assimilation of iron by diatoms, negative oxidation-reduction potentials and values of pH as low as 5 may well arise. The maximum activities of ferrous ion which may exist in equilibrium with ferric ion under a variety of conditions are therefore set out in Table IV.

The activity of ferrous ion which can exist in equilibrium with saturated ferric ion at certain reversible and irreversible oxygen potentials is given in Table V. These potentials, reversible or irreversible, are those set up by oxygen in equilibrium with air, at 1 atm. pressure, dissolved in water at the values of pH given in the table. The great increase in the activity with small changes in pH and E^h is clearly brought out. In Table VI are summarized the activities of the three ions, Fe^{+++}, $FeOH^{++}$, and Fe^{++} which will be present in equilibrium in sea water, containing solid ferric hydroxide.

As regards the ferrous ion the significance of the reversible and irreversible potentials was discussed in a previous paper (Cooper 1937). If, for example, a small amount of a ferrous salt were added to sea water at *p*H 8·0 (this *p*H we will assume to remain unchanged), the ferrous ion would be rapidly oxidized and ferric hydroxide would be precipitated until about 10^{-9} mg.-atom Fe^{++} per cubic metre was left in solution, corresponding to the irreversible oxygen potential. Further oxidation might then proceed more and more slowly until after an infinite time the activity would reach

TABLE IV—MAXIMUM ACTIVITIES OF FERROUS ION, AS MG.-ATOMS PER CUBIC METRE, WHICH CAN EXIST IN EQUILIBRIUM WITH A SATURATED SOLUTION OF FERRIC ION

E^h	*p*H ... 8·5	8·0	7·5	6·0	4·5
+0·8	10^{-16}	$10^{-14\cdot5}$	10^{-13}	$10^{-8\cdot5}$	$10^{-3\cdot9}$
+0·6	$10^{-12\cdot5}$	10^{-11}	$10^{-9\cdot5}$	10^{-5}	0·4
+0·4	10^{-9}	$10^{-7\cdot5}$	10^{-6}	0·04	1000
+0·2	$10^{-5\cdot5}$	10^{-4}	0·003	100	3×10^{6}
0·0	0·008	0·3	9	3×10^{5}	Sat.
−0·2	25	800	25,000	Sat.	Sat.
−0·4	65,000	Sat.	Sat.	Sat.	Sat.

"Sat." denotes that under the conditions stated the solubility product of ferrous hydroxide would be exceeded.

TABLE V—SUMMARY OF FERROUS ION ACTIVITY AS CONTROLLED BY THE REVERSIBLE AND IRREVERSIBLE OXYGEN POTENTIALS AT DIFFERENT VALUES OF *p*H

Values of *p*H mg.-atoms Fe per cubic metre

	Reversible		Irreversible	
*p*H	E^h volt	$a_{Fe^{++}}$	E^h volt	$a_{Fe^{++}}$
8·5	0·73	2×10^{-15}	0·44	1×10^{-10}
8·0	0·76	2×10^{-14}	0·46	$1\cdot5 \times 10^{-9}$
7·5	0·79	2×10^{-13}	0·49	2×10^{-8}
6·0	0·88	2×10^{-10}	0·56	4×10^{-5}
4·0	0·99	2×10^{-6}	0·66	1

TABLE VI—SUMMARY OF MAXIMUM ACTIVITIES OF DIFFERENT FORMS OF IONIC IRON IN SEA WATER; MG.-ATOM Fe PER CUBIC METRE

			$a_{Fe^{++}}$	
*p*H	$a_{Fe^{+++}}$	$a_{FeOH^{++}}$	Governed by $a_{Fe^{+++}}$ and reversible oxygen potential	Governed by $a_{Fe^{+++}}$ and irreversible oxygen potential
8·5	4×10^{-16}	5×10^{-10}	2×10^{-15}	1×10^{-10}
8·0	$1\cdot3 \times 10^{-14}$	5×10^{-9}	2×10^{-14}	$1\cdot5 \times 10^{-9}$
6·0	$1\cdot3 \times 10^{-8}$	5×10^{-5}	2×10^{-10}	4×10^{-5}

10^{-14} mg.-atom per cubic metre, corresponding to the reversible potential. From the nature of the oxygen potential it is not possible to deduce much more than this and the expression "equilibrium" must be used with care. Even so it is of much value to know the limits within which the ferrous-ion activity is likely to lie.

In the above treatment we have assumed that the only ionic forms of iron likely to be present are Fe^{+++}, $FeOH^{++}$, and Fe^{++} and that their activities in sea water will be as given in Table VI. Finally we must consider the work of Jander and Jahr (1936) and their collaborators on the existence of ferrichloric acids in solutions of certain ferric salts. They examined diffusion coefficients in solutions of ferric perchlorate and of ferric chloride and were able to show that ferric perchlorate behaves as a true salt. Its behaviour justifies the treatment which Bray and Hershey gave to electrochemical measurements made with it in order to evaluate K_1° for the reaction involving $FeOH^{++}$ (equation (2), p. 300). The inability of the perchlorate ion to enter into complex formation through co-ordination linkages follows from the absence of free electron pairs in the central chlorine atom. The chloride ion is not limited in this way, so that the behaviour of solutions of ferric chloride is quite different. Jander and Jahr (1936) suggest that in strongly acid solution ($p\text{H} < 3$) the compound $H_3[FeCl_6]$ is present, and hydrolysis of this by increasing additions of alkali can be described schematically by the equations:

$$H_3[FeCl_6]\,\text{aq.} + NaOH \rightleftharpoons H_3[FeCl_5(OH)]\,\text{aq.} + NaCl,$$

$$H_3[FeCl_5(OH)]\,\text{aq.} + NaOH \rightleftharpoons H_3[FeCl_4(OH)_2]\,\text{aq.} + NaCl.$$

Although there is no evidence as to what conditions govern the final stage of this hydrolysis

$$H_3[FeCl(OH)_5]\,\text{aq.} + NaOH \rightleftharpoons H_3[Fe(OH)_6]\,\text{aq.} + NaCl,$$

there is no reason to believe that ferrichloric acids can exist in sea water at pH 8.

In solutions of lower pH containing chloride ion the behaviour of iron may well be affected in some such way as this. Bray and Hershey (1934) also produced some evidence for the existence of the ion, $FeCl^{++}$; if their calculation may be extended to pH 8—and this appears doubtful—the activity of $FeCl^{++}$ will be between ten and twenty times that of Fe^{+++}, so that, compared with $FeOH^{++}$ and Fe^{++}, $FeCl^{++}$ may be ignored.

All the evidence presented in this paper deals with equilibrium conditions, and there is evidence that equilibrium in the iron system is but slowly attained. Even so, in the sea we are concerned with a time scale measured in weeks or months and not in minutes as in most laboratory experiments, so that it is not unreasonable to conclude that the results of this investigation give a good general picture of events as they occur in the sea.

The treatment is general and applies not only for sea water but also for other natural waters and for physiological fluids whose oxidation-reduction potentials are controlled by dissolved oxygen. In any case the maximum activities of Fe^{+++} and $FeOH^{++}$ will be independent of oxidation-reduction potential. For any system of known E^h and pH, the ferrous-ion activity at equilibrium may be found from Table IV or equation (13).

SUMMARY

Existing data on the solubility products of ferrous and ferric hydroxides and on the ion, $FeOH^{++}$, have been used to calculate the maximum activities of Fe^{++}, $FeOH^{++}$ and Fe^{+++} which may exist in sea water.

The maximum activity of ferrous ion is controlled both by the activity of the ferric ion and by the oxidation-reduction potential of the system.

The maximum activity of the three ions at equilibrium in sea water are presented in Table VI. Other tables show equilibrium conditions at a range of oxidation-reduction potentials and hydrogen-ion concentrations.

The total quantity of iron in true solution in water after equilibrium has been attained does not exceed

3×10^{-8} mg. iron per cubic metre at pH 8·5
4×10^{-7} ,, ,, ,, 8·0
4×10^{-5} ,, ,, ,, 7·0
5×10^{-3} ,, ,, ,, 6·0

and of this the greater part consists of ferrous and $FeOH^{++}$ ions. In more acid solutions ferrichloric acids may have to be taken into account.

These values apply to iron in sea water, in natural waters and in many physiological fluids.

REFERENCES

Bray, W. C. and Hershey, A. V. 1934 *J. Amer. Chem. Soc.* **56**, 1889.
Britton, H. T. S. 1925*a* *J. Chem. Soc.* **127**, 2110.
— 1925*b* *J. Chem. Soc.* **127**, 2148.
Cooper, L. H. N. 1935 *Proc. Roy. Soc.* B, **118**, 419.
— 1937 *J. Mar. Biol. Ass.* N.S. **22** (in the Press).
Jander, G. and Jahr, K. F. 1936 *Kolloidbeihefte*, **43**, 295.
Jellinek, K. and Gordon, H. 1924 *Z. phys. Chem.* **112**, 207; *Chem. Abs.* **19**, 1520 (1925).
Kriukov, P. A. and Awsejewitsch, G. P. 1933 *Z. Elektrochem.* **39**, 884.
Ruff, O. and Hirsch, B. 1925 *Z. anorg. Chem.* **146**, 388.
Whitman, W. G., Russell, R. P. and Davis G. H. B. 1925 *J. Amer. Chem Soc.* **47**, 70.

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Electrotonus and Excitation in Nerve

By H. Rosenberg

From the Departments of Physiology, University College, London, and London Hospital Medical College

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I—Introduction

The peculiar relations between electrotonic and excitatory processes lead to the fundamental problem: is the establishment of catelectrotonus (or the disappearance of anelectrotonus) responsible for the development of the change in nerve which culminates in excitation?

It has been suggested, without direct evidence, that a certain shift of the membrane potential by means of polarization or charge of the axon membrane is the physical condition for the occurrence of the physiological reaction in electrical stimulation (Lapicque 1907; Bernstein 1912; Ebbecke 1927). The core-conductor properties of nerve, however, permit the observation, in the polar region, of a possible parallelism between the magnitude of such potential changes at any moment and the onset of the electrical response (Cremer 1932 *a*). The physico-chemical basis of this conception has recently been reviewed (Rosenberg 1937). An extended core-conductor scheme is shown in fig. 1, including the longitudinal resistances of both core r_4 and envelope r_3, and the transverse resistance r_2 (myelin sheath (?), i.e. a substrate of longitudinal resistance much greater than r_3) situated in front of the polarizable interface (or static membrane capacity) which is represented by condenser c with leak resistance r_1.

The consequences of the theory were experimentally investigated by Rosenberg (1932). It is possible to record the development of ion accumulation, regardless of the type of capacity involved, during the action of the electrical stimulus at the stimulated point (or at a lateral distance from it—electrotonus in the usual terminology) by leading-off the electrotonic potential difference between the pole (or an adjacent extrapolar point) and a distant unaffected portion of the nerve. The shape and rate of rise of electrotonus generated by a subliminal direct current pulse were shown to resemble the features of Cremer's (1932 *b*) theoretical curve for the establishment of polarization in nerve, particularly at a lateral distance from the pole.

To a first approximation, however, the major part of the rise (and fall) of the electrotonic potential at the pole may be regarded as exponential (Bogue and Rosenberg 1934). In the sciatic nerve of *R. temporaria* 1/2 and 1–1/*e* of the maximum of the catelectrotonic potential are reached 0·17 and 0·25 msec. after make of a direct current, and practically the final value is attained after about 3 msec. at room temperature. At threshold stimulation with direct current the action potential appears at the cathode when the electrotonic rise has almost reached its maximum. If we take about 1/100 of a maximum action potential as a threshold response, the time interval between make of the current and start of the electrical response is about 1·85 (1·7–2·0) msec. at 18° C. (these nerves were kept at least 1 hr. in saline with 0·01 % $CaCl_2$, 0·0075 % KCl and 0·01 % $NaHCO_3$ which probably did not alter their natural accommodation (see Solandt 1936; unpublished experiments by Rosenberg 1929)). According to Blair and Erlanger (1936) the time interval between make and response at the excited point corresponds to the utilization time in threshold stimulation by direct current pulses of rheobasic strength. Hence the conclusion is justified that under these conditions excitation occurs when the rise of the cathodic potential is essentially finished. The final value is about 30 % of the applied voltage (measured between the contact points of the electrodes). Since this external rheobasic voltage is of the order of 30 mV in the sciatic of *R. temporaria* at 1 cm. electrode distance, the internal threshold is about

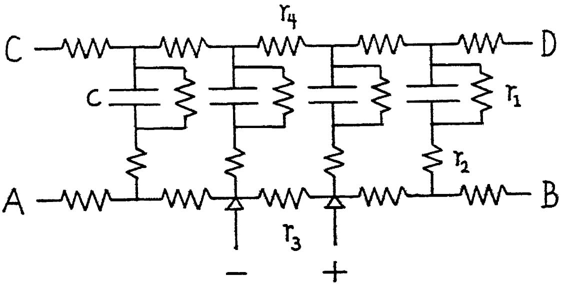

FIG. 1—Scheme of a resistance-capacity conductor completed by resistances of core and sheath. *AB*, envelope; *CD*, core; *c*, membrane (? interface) capacity; r_1, membrane (leak) resistance; r_2, myelin (?) sheath resistance; r_3, envelope (connective tissue+interstitial fluid) resistance; r_4, core (axon) resistance; −, +, leading-to electrodes. *c*, r_1 and r_2 constitute Hermann's equivalent circuit for a single polarizable element.

9 mV (determined on the trunk). In threshold stimulation by condenser discharges the peak height of the catelectrotonic potential is identical when the voltage is doubled and the capacity readjusted for minimum response (Rosenberg 1931).

Schmitz and Schaefer (1933, 1934 *a, b*) obtained similar results which they interpreted in terms of a voltage theory of stimulation (see also Schaefer 1936 *a*). This conception was supported by the observation that in threshold stimulation with relatively slow condenser discharges (*RC* of the order of 1–2 msec.) the action potential started when the electrotonic potential at the cathode passed through its maximum (the discharges being distorted by the nerve reactance). In accordance with Cremer's (1932 *b*) assumption they stated that the half-time of the catelectrotonic rise at make of a direct current was of the order of ordinary chronaxie values, and supposed that the action potential originated half-way during the rise when a direct current of double the rheobasic strength was applied. Since in these circumstances the start of the action potential was indistinct, they measured the duration of the rising phase of a propagated wave elicited by the same stimulus, and deducted this duration from the interval between make and summit of the wave recorded at the cathode. Such a stimulus, however, will affect numerous fibre groups in succession, and the propagated wave will show an enhanced temporal dispersion. These and other inconsistencies (e.g. at double the rheobasic voltage the catelectrotonic potential was not doubled) invalidate the argument. Actually in his last investigation Schaefer (1936 *b*) encountered great difficulties in comparing half-time of catelectrotonic rise and individual chronaxie in the same preparation (see § IV).

"Threshold" responses of nerve to alternating current, simultaneously with the stimulating current or the electrotonic potential, have been recorded at low and medium frequencies after conduction (Lullies 1930 *c*; Schaefer and Göpfert 1936). It appears that the response occurs at medium frequencies on the crest and at low frequencies during the rise of the negative half-cycle of the electrotonic potential at the respective pole. (With decrease of frequency the accommodation of nerve to the electric stimulus requires the onset of excitation to regress increasingly from the catelectrotonic crest, but noticeably only below the optimum frequency (Hill 1936 *a*; Hill, Katz and Solandt 1936).)

On the basis of all these observations we may express the hypothesis in the precise form that excitation occurs if and when the internal threshold is reached by the catelectrotonic potential in the course of its development. Since threshold variations are negligible over a wide range of durations and frequencies, it is admissible to ignore accommodation and to regard the threshold condition as constant within this range. We can, therefore, discard the difficult absolute measurement of electrotonic potential at minimum electrical response and confine our observations to an arbitrarily fixed subliminal catelectrotonic potential.

Hence, if excitation is determined by the rise of the catelectrotonic potential to a critical value, then all the relations (strength duration, voltage capacity, intensity frequency, and their modifications with interelectrode distance, etc.) which have been found for threshold excitation should have their counterpart in the rise of the electrotonic potential to a given subliminal value. Instead of taking threshold excitation as index and measuring the voltage required for a given capacity, or the intensity required at a given frequency, the rise of electrotonic potential to a fixed subliminal height may be taken as index and the relation between voltage and capacity, or intensity and frequency determined (strength-duration curves were not recorded because of the technical difficulties in the precise generation of extremely short rectangular shocks). Graphical representation permits a comparison of the results obtained in electrotonus with theoretical curves which fit the data obtained in excitation (Hill 1936 *a*, *b*; Hill, Katz and Solandt 1936). In this way the analogies between electrotonus and excitation, with regard to shape and time constant of the curves, were investigated in medullated nerve. The results of preliminary experiments have been communicated in brief by Harris and Rosenberg (1935) and by Harris, Rosenberg and Sager (1935).

II—METHODS

Amplifier—Three units built according to the balanced bridge scheme (see Harris 1932) were coupled directly. This design ensured great stability of base-line and good constancy of amplification. When balance was established the input valve was operated with free grid potential; hence no grid current passed through the nerve connected with grid and cathode (= proximal earthy lead). Sensitivity and linearity were regularly checked in the course of observations by means of D.C. potentials, 1 mm. deflexion on the screen of the cathode-ray tube corresponding with 0·15–0·18 mV. The frequency characteristic of the amplifier began to slope at 1600 hz. (hertz = cycle per sec.), but the decline was marked only beyond 3000 hz. (about 10 % at 3200 hz.).

Hence in experiments on A.C. electrotonus usually the frequency of 3000 hz. was not exceeded. This deficiency of the amplifier, however, does not impose a strict upper limit in practice, provided that both polarizing and electrotonic potentials are measured after amplification. As will be shown, the subthreshold electrotonus generated by sinusoidal currents and condenser discharges (similar to that by direct currents) shows a linear potential summation when the polarizing potential is raised in steps. If on account of the decline in frequency response of the amplifier the polarizing potential has to be increased in order to produce a finite electrotonic potential, the relative errors of potential measurements are the same in the two cases and, therefore, do not affect the result. This method seems safe within reasonable frequency limits.

Condenser discharges—Inductance and stray capacity of the simple circuit adopted for the delivery of single condenser discharges were not measured; but since the wiring was very similar to that used in previous experiments the same negligible values may be accepted without serious error (Rosenberg 1935). The polarizing and recording circuits were entirely insulated from ground and—connected through the adjacent electrodes—kept at local earth potential. This arrangement fulfilled the condition of a minimum current spread in dead nerve (in order to detect such errors a frequent control on dead nerves was observed).

With single discharges the reading, particularly of the very rapid deflexions, is rather difficult, but still practicable with some precautions. Since, for simplicity, no sweep was provided, only the vertical movement of the spot was observed between two fine horizontal lines on the screen for marking base-line and peak height of the finite electrotonic potential change. Precision was conveniently obtained by adjusting subsequent deflexions just below, level with, and just above the top mark. Assuming that with a steady spot differences of 0·25 mm. are discernible, the error is less than ± 1 % when the full deflexion is 25 mm. The error increases, of course, if extremely rapid discharges are applied and/or the deflexions are diminished in height.

For a given voltage E the discharge times RC were determined which gave the same electrotonic potential when R was made large or small. The close agreement of the observed values confirmed that the results were not vitiated by inductance or capacity of the circuit. When examined with time base the discharges showed no trace of oscillation. In order to avoid the uncertainty of faint deflexions and a critical transgression of the frequency limit of the amplifier, the shortest discharge times used were of the order of 20 μsec.

Electrodes, chamber, preparation—The electrodes were calomel half-cells of large mercury surface which prevented any polarization for the duration and strength of the applied currents. The D.C. resistance of a pair, made up with Ringer's solution, was less than 2000ω.

The nerve was placed in a paraffin-wax chamber upon the edges of moistened filter-paper strips (width of contact about 0·5 mm. in the longitudinal direction of the nerve). The chamber was fitted with five strips and seven electrodes (fig. 2). By suitable combination of leading-to and leading-off electrodes, the electrotonic potentials could be determined at the pole itself or at a variable extrapolar point (i.e. with the "intermediate" distances 0, 1·5, 3 mm.) with both long or short polarizing length ("interpolar" distances). Before and after use the absence of leakages between the strips was checked. The resistances of nerve plus electrodes and strips were measured with a D.C. bridge.

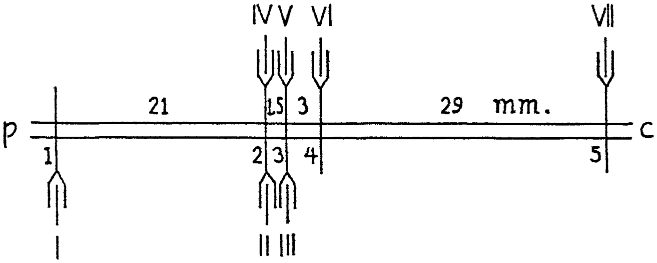

FIG. 2—Electrode arrangement. 1–5, filter-paper strips (distances between middles inserted in diagram); I–III, leading-to electrodes; IV–VII, leading-off electrodes (VII permanently connected with grid lead; IV, V, VI alternatively connected with cathode lead). The length of nerve between adjacent leading-to and leading-off electrodes is the "intermediate" distance. *p*, peripheral; *c*, central end of nerve bridging the filter-paper strips.

The sciatic nerves of Hungarian frogs were dissected with an unbranched peripheral part as long as possible and tied at both ends (total length 65–70 mm.). After soaking for about 1 hr. in saline (NaCl 0·6 %, $CaCl_2$ 0·01 %, KCl 0·0075 %, $NaHCO_3$ 0·01 %) at room temperature, the nerve was mounted in the chamber with the peripheral end on strip 1 in order to utilize the unbranched part for the polarized and adjacent electrotonic region. Between the leading-off electrodes injury potentials, if present at all, were very small. The experiments were made at room temperature.

Alternating currents—For the generation of sine waves two heterodyne oscillators were available. An experimental type, constructed by Professor D. T. Harris, covered a range from 50 to 3200 hz. with a good wave form and even output over the major part of the band. The frequency was calibrated and retested during the experiment with a commercial type

(Birmingham Sound Reproducers Ltd., battery-operated model) with two frequency scales from 10 to 15,000 hz. which were adjusted by zero-beat control. According to specification the harmonic content was less than 2 % of fundamental down to 20 hz. Most of the experiments were made with the experimental oscillator because it gave practically no electrical interference. With small output (by transformer with 5000 ω impedance) the commercial oscillator also gave satisfactory results and was used when the observations were extended to lower and higher frequencies. Unfortunately the low-frequency scale was not sufficiently subdivided.

The peak voltage applied to the electrodes and the peak current (P.D. across a small non-reactive series resistance) sent through the nerve from a non-reactive potential divider were measured with amplifier and oscillograph after adjustment of the catelectrotonic peak potential to index height.

The electrical conditions secured the absence of a substantial spread at a small distance from the pole in dead nerve, even with relatively high frequencies, when voltages were applied which were equal to those used for the measurement of electrotonus at the pole in living nerve. For instance, at an intermediate distance of 1·5 mm. (2 mm. between the middles of the filter-paper strips) with 2400 hz. the potential in a dead nerve was about 1 % of the electrotonic potential in a living nerve at 0 mm. intermediate distance and below 0·5 % with medium frequencies. With low frequencies at 0 mm. distance the potential in dead nerve was about 1/10 of that in living nerve; this ratio, however, rose noticeably with increase of frequency. Possibly balanced output of oscillator and input of amplifier might be suitable to overcome this difficulty. Some experiments were made, under different conditions, with an A.C. amplifier of less than 2 % loss at 20 hz. (from the Research Laboratories, Marconi Works, Chelmsford), which gave essentially the same results as those with the D.C. amplifier.

III—RESULTS

A—*Condenser discharges*

All the experiments concern catelectrotonus, since anelectrotonus is of no immediate importance in stimulation; the classical statement (Cremer 1929) that only genuine cathodes stimulate could be supported by special experiments with condenser discharges.

The relation between the potential E and the time constant RC of a condenser discharge in threshold stimulation is expressed by the equation

$$E/E_0 = (RC/k)^{\frac{1}{RC/k-1}} \tag{1}$$

(see Hill 1936 *a*). E_0 is the rheobasic potential and k is the time constant of excitation or—in the proposed interpretation—of rise of the electrotonic potential at the stimulating cathode. Although this formula disregards accommodation, it is valid for medullated nerves of frogs over a ten-thousandfold range of discharge times (see Hill 1936 *b*). It also applies to medullated mammalian nerves as is indicated by a curve (fig. 3) which is plotted from the mean results obtained in dog's phrenic nerves (Rosenberg 1935): the predicted minimum quantity of electricity required for excitation with discharges of extremely short durations is closely approximated. It seems, therefore, justified to base the comparison between electrotonus and excitation upon this equation, without considering, for the time being, its theoretical foundation.

Following Rushton's (1931) suggestion the observed values for E and RC were plotted on a bilogarithmic scale (for accuracy the scale of the voltage ordinate was taken twice that of the time axis). This representation eliminates the variability in shape with E_0 and k and thus simplifies enormously the comparison with the theoretical curve and the exact location of the point of minimum energy where $RC = k$. To this end Hill's (1936 *b*) procedure was adopted.

The diagrams obtained in this way show the theoretical curves drawn through the experimental points from which time constant and rheobase are derived. It may be anticipated in general that, regarding shape, the coincidence between observed and calculated curve is perfect within the limits of error for the range of discharge times investigated. Although the curves are not continued towards the shortest durations, it seems admissible to infer that the curves tend towards a minimum quantity (see fig. 4). The points of minimum energy are marked by arrows and the rheobases are indicated by lines on the right side of the diagrams. The absolute value of E_0 has, of course, no other meaning than to designate a subthreshold potential appropriate to generate an arbitrary fixed catelectrotonic potential P.

1—*The time constant as a function of intermediate distance.*

The results of a set of measurements are summarized in Table I. Omitting one experiment, we obtain on an average 185 μsec. for k at the cathode, i.e. with intermediate distance 0 mm., the mean interpolar length being about 20 mm. (see fig. 2). If we assume that under these conditions the rise of catelectrotonic potential at make of a direct current is exponential, the half-time of the rise, calculated from a time constant of 185 μsec., amounts to 130 μsec. This value is near the lower limit of the half-time

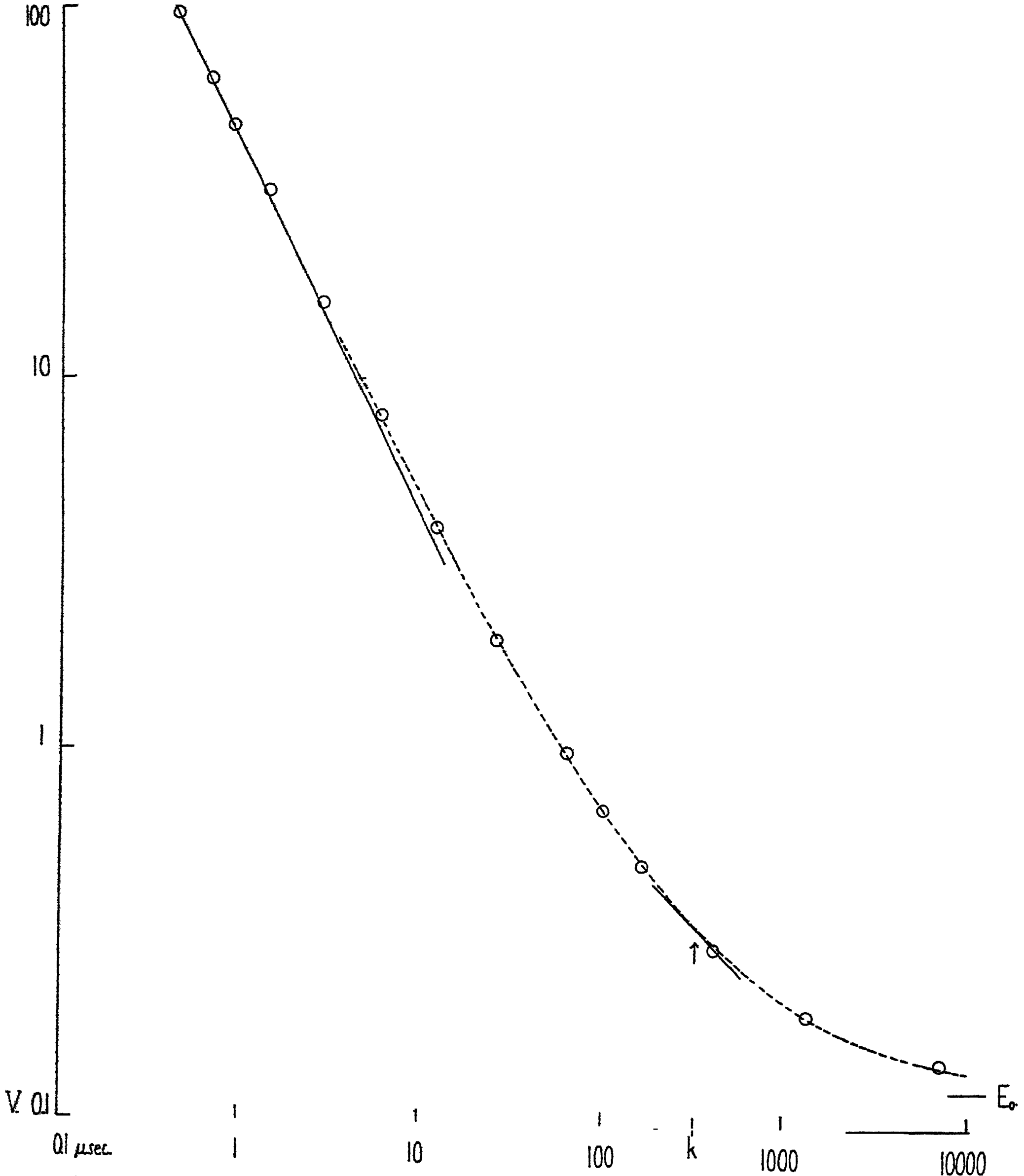

FIG. 3—Voltage-capacity curve of dog's phrenic nerve for semimaximal stimulation by single condenser discharges. Average curve from four experiments. Mean temperature 21° C. Circles, observed points; broken line, theoretical curve from equation (1); continuous line, asymptote for minimum quantity of electricity; arrow, contact point of tangent for minimum energy where $RC = k = 327$ μsec.; extrapolated rheobase (horizontal line), 0·126 V, i.e. about 0·1 V between points of contact on nerve, for semimaximal response.

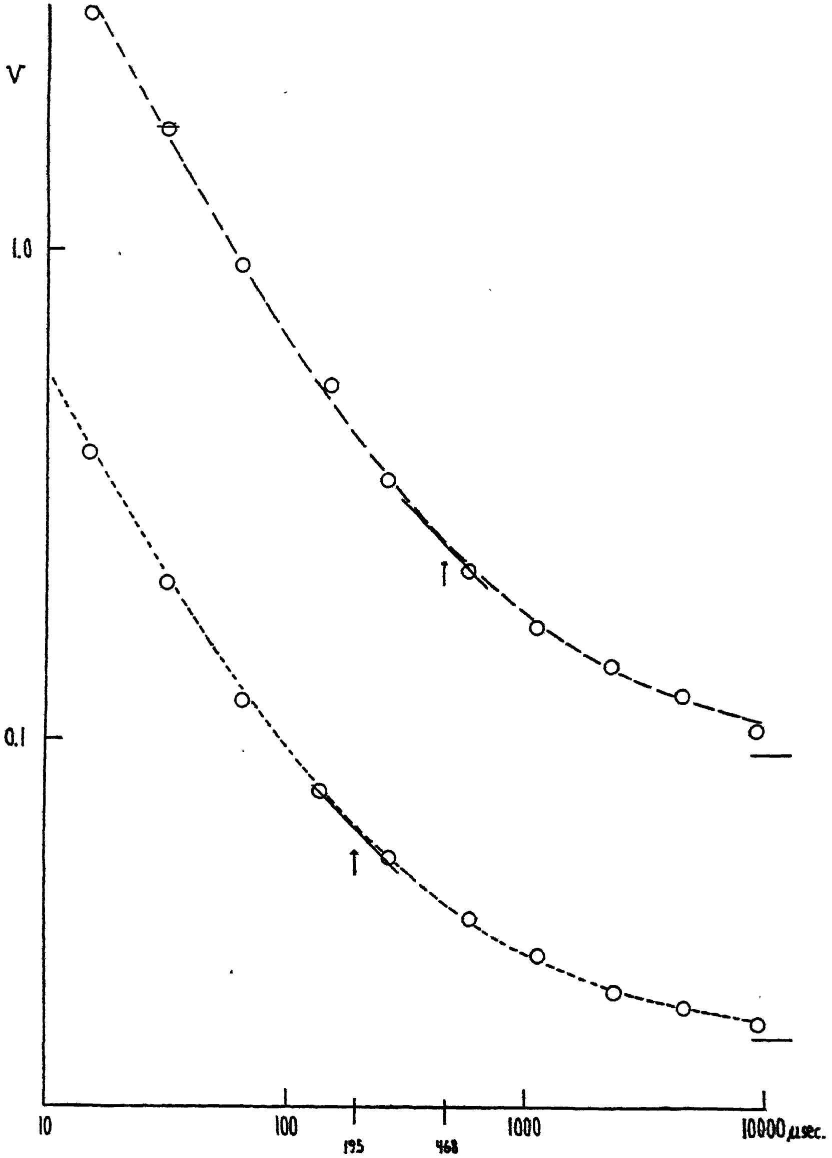

FIG. 4—Voltage-capacity curve for constant catelectrotonic potential with intermediate distances 0 and 3 mm. Notation as in fig. 3. Sciatic nerve of Hungarian frog at room temperature (19·4° C.).

Curve	Interpolar distance mm.	Intermediate distance mm.	Catelectrotonic potential mV	k μsec.
Lower	22·5	0	6	195
Upper	22·5	3	3	468

data (124–212μsec.) directly determined in sciatics of *R. temporaria* (Bogue and Rosenberg 1934).

TABLE I—ELECTROTONIC TIME CONSTANT k, "RHEOBASE" E_0 AND POTENTIAL P FOR INTERMEDIATE DISTANCES 0 AND 3 MM. INTERPOLAR DISTANCE 16–22·5 (AVERAGE 19) MM.

	k, 10^{-6} sec.		P, 10^{-3} V		E_0, 10^{-3} V		P/E_0 %	
° C.	0 mm.	3 mm.	0 mm.	3 mm.	0 mm.	3 mm.	0 mm.	3 mm.
16·7	162	562	5·71	5·71	27·9	120	20·5	4·75
16·8	178	398	5·85	2·93	35·1	105	16·7	2·80
19·4	195	468	6·00	6·00	24·6	94·4	24·4	6·35
16·9	170	427	6·80	3·40	26·3	110	25·8	3·10
18·5	200	468	6·00	3·00	33·5	89·1	17·9	3·36
19·3	193	—	6·00	—	27·5	—	21·8	—
18·0	195	—	6·55	—	28·2	—	23·2	—
Av. 18·0	185	465	6·13	4·21	29·0	103·7	21·5	4·07
19·0	157	316	6·56	3·28	36·7	219	17·9	1·50
19·2	—	376	—	2·40	—	136·5	—	1·76

With the commutator technique (see Hill 1934; Harris and Rosenberg 1935) voltage-capacity curves were recorded for "minimum" electrical response at the stimulated point in nerves of the same batch of Hungarian frogs as used in the observations of electrotonus. The time constant k for excitation was about 360μsec. at room temperature, in accordance with the ordinary values quoted by Hill (1936 *b*). It is obvious, therefore, that the electrotonic k is only about half the excitatory k.

A possible explanation was the supposition that the electrotonic time constant might increase when the electrotonic potential was raised, and approach the excitatory time constant when the threshold was attained. Beside the general interest in the relationship between magnitude of the polarizing potential and development (and height) of the electrotonic potential, a special interest was attached to the problem because of an experimental difficulty. With a polarizing potential giving an electrotonic deflexion over the whole linear range of amplification when led-off at the cathode, the deflexion was insufficient for an exact measurement when led-off at intermediate distances of 2–3 mm. This well-known decline of electrotonic potential along the extrapolar region made it necessary to increase the polarizing potential E considerably in order to obtain a suitable deflexion P. Now a comparison between electrotonic time constants k and between electrotonic potentials P at different intermediate distances would be admissible only if (1) k were independent of E and P (subthreshold), and (2) E and P were in a simple relation.

For these reasons voltage-capacity curves were recorded at the cathode for two different electrotonic deflexions, for convenience one twice the other (40 and 20 mm. equal to 6 and 3 mV). The corresponding data were plotted, their time constants and relative rheobases computed, and the ratios P/E_0 calculated for the two cases. It appears that there is no increase of k with enhancement of the electrotonus, both curves giving the same value. Exactly double the "rheobasic" voltage is required for generation of double the electrotonic potential. The same relation exists between the voltage pairs for the respective discharge times. Accordingly, the ratios P/E_0 are identical within the limits of error (e.g. 25·8 and 25·4 %). We conclude, therefore, that in electrotonus (1) k is independent of E and P, and (2) P obeys a law of summation over the whole range from shortest discharges to "rheobasic" conditions, at least for the applied subthreshold potentials.

This result, of course, fulfils the theoretical predictions for a core-conductor model composed of fixed resistances and capacities. It was, however, by no means certain that the membrane capacity (or capacities) would not be affected by an increase of polarizing potential in the same manner as that of a non-corrodable electrode when the critical voltage is exceeded. Recently, Schaefer (1936 *b*) observed proportionality between E and P for long rectangular pulses. The same relationship between intensity of a continuous polarizing current and the electrotonic current (with intermediate length of several mm.) was supposed already by Du Bois-Reymond (1849), but was difficult to establish with inert instruments because of the gradual change in time of both magnitudes.

When the development of electrotonic potential generated by a long subliminal pulse is recorded at different lateral distances from the pole, an approximately linear relation is found between the duration of the rise to a definite fraction of the maximum and the intermediate distance (Bogue and Rosenberg 1934). These observations suggested an increase of the electrotonic k when the voltage-capacity curves were determined at extrapolar points. For technical reasons curves were obtained only for one intermediate distance different from zero, namely 3 mm. (measured between the middles, i.e. 2·5 mm. between the inner edges of the strips). Contrary to theoretical expectation (see § IV) the observed points fit the calculated curve excellently (fig. 4). The position of the curve is shifted towards longer durations and higher voltages. The numerical results obtained in the same nerve at 0 and 3 mm. intermediate distance are: k, 195 and 468 μsec.; E_0, 0·0246 and 0·0944 V; P/E_0, 24·4 and 6·35 % respectively. Although 3 mm. lateral from the cathode the electrotonic rise at make of a direct current is slightly S-shaped, we may, with due caution, compare its duration of rise to 1–1/*e* of the final height with the time constant derived from the electrotonic voltage-capacity curve. We infer from the previous measurements on sciatics of *R. temporaria* that the present electrotonic k at 3 mm. is shortened by approximately the same ratio as k at 0 mm. intermediate distance. This concord is essential since, as proved by tests in dead nerve, the extrapolar electrotonus is certainly not vitiated by any physical current spread. The mean value of k at 3 mm. computed from a series of such experiments is 465 μsec. (see Table I). The last two experiments are not included in the average because, at 3 mm. intermediate distance, they yield a very low ratio P/E_0 associated with a relatively small k (the second experiment of the series shows a similar tendency).

In this connexion we note that P/E_0 measured at the cathode is smaller in sciatics of Hungarian frogs than in those of *R. temporaria* (17–26 % as compared with 30–40 %). The threshold potentials for direct current are also different, viz. greater in the nerves of Hungarian frogs than in those of *R. temporaria*, whereas, as has been shown, the electrotonic time constant at the pole is smaller in the former than in the latter.

It seems possible to reduce these various differences formally to a common factor if we assume that the equivalent of the parallel resistance r_1 of the core-conductor circuit (fig. 1) is (absolutely or relatively) smaller in one type of nerve (Hungarian frog) than in the other. Obviously the fraction of the applied voltage to which the polar (membrane) capacity c is charged diminishes with decrease of r_1; at the same time the duration of the charge to, say, 1–1/*e* of the final voltage is shortened (Hermann 1905). Accordingly, the temporal rise of extrapolar electrotonic potential is accelerated while the gradient of its spatial decline is increased. Hence the smaller r_1 the steeper the fall of P/E_0 along the nerve, i.e. in order to obtain the "original" final potential difference across c at any point the applied voltage has to be raised according to the diminution of r_1 and to the intermediate distance, while the corresponding "time constants" appear to be lowered.

2—*The time constant as a function of the interpolar distance.*

The chronaxie of frog's nerve decreases (by about 35 %), and the rheobasic voltage increases when the distance between the stimulating electrodes is reduced from 12 to 2 mm. (Cardot and Laugier 1914). The increase of threshold intensity of a direct current in these circumstances is a fact previously established by several workers. It is interpreted by the hypothesis

(Rushton 1934) that under constant threshold conditions the same current has to traverse the membrane at the cathode with any interpolar length, and that the increase of total current with decrease of the interpolar distance is necessitated by a corresponding diminution of the penetrating fraction, this change in current distribution depending on the resistance conditions in a core-conductor. Recently it has been demonstrated (Bouckaert and Katz 1935; see also Hill 1936 *b*) that the voltage-capacity curve maintains its shape while the time constant decreases to nearly a half when the interpolar distance is diminished from 15–21 mm. to less than 1 mm.

In order to elucidate how far changes of k in electrotonus might explain the diminution of k in excitation, voltage-capacity curves were recorded for different interpolar and intermediate distances. The cathode was kept in a fixed position and the anode was changed between 1·5 and 22·5 mm. distance; the intermediate distance was alternately 0 and 3 mm. The results of an experiment are represented in fig. 5 which shows that the experimental data satisfy the theoretical curves in all the conditions. With 0 mm. intermediate distance the electrotonic k was reduced from 200 to 141 μsec. (i.e. by 29·5 %) and with 3 mm. intermediate distance from 468 to 327 μsec. (i.e. by 30·1 %), when the interpolar distance was diminished from 22·5 to 1·5 mm. At the same time the relative "rheobases" decreased from 33·5 to 16·4 mV and from 89·1 to 63·1 mV respectively, while the ratios P/E_0 increased from 17·9 to 35·1 % and from 3·36 to 4·75 % respectively. In dead nerve with intermediate distance 0 mm. the spread also increased when the interpolar length was reduced, whereas with 3 mm. intermediate distance there was no spread at all for the capacities and voltages used in the living nerve. Hence the increase of spread at 0 mm. intermediate distance has no decisive influence on the result, and the decrease of both time constant and "rheobase" in electrotonus is real. The mean values of four experiments at 22·5 and 1·5 mm. interpolar distance are for k, 183 and 144 μsec. (i.e. 21·3 %) and 387 and 288 μsec. (i.e. 25·5 %) at 0 and 3 mm. intermediate distance respectively (some of the nerves showed low ratios P/E_0 and accordingly small values of k at 3 mm. intermediate distance).

While there is a complete analogy between the time constants of electrotonus and excitation in their dependency upon interpolar length, the behaviour of the electrotonic "rheobase" seems to differ from that of the excitatory rheobase observed by the workers quoted. This discrepancy is probably not essential, since the voltage largely depends on circuit conditions if it is not measured directly between the points of contact on the nerve. From the voltages and resistances observed the currents passed

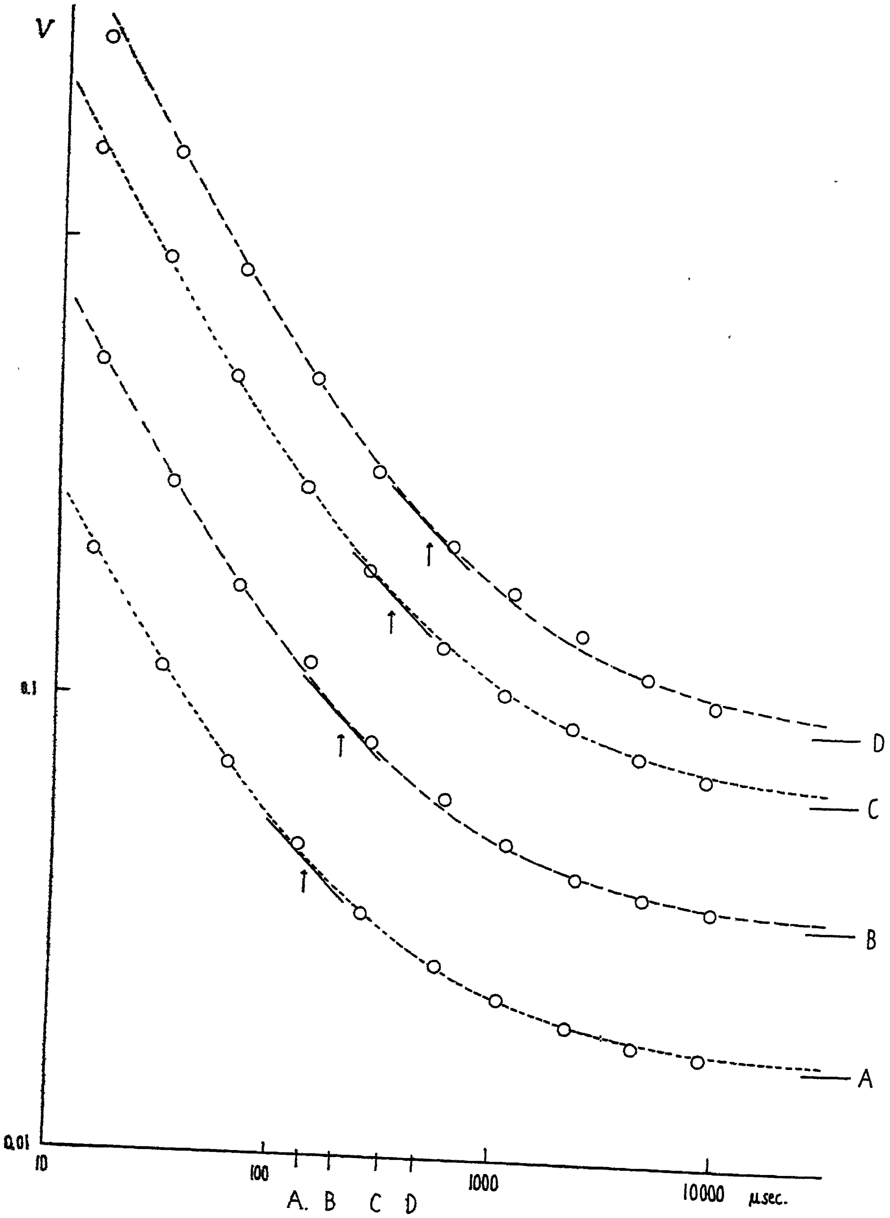

FIG. 5—Voltage-capacity curve for constant catelectrotonic potential (*a*) with interpolar distances 1·5 and 22·5 mm., and (*b*) with intermediate distances 0 and 3 mm. Notation as in fig. 3. Sciatic nerve of Hungarian frog at room temperature (18·5° C.).

Curve	Interpolar distance mm.	Intermediate distance mm.	Catelectrotonic potential mV	k µsec.
A	1·5	0	6	141
B	22·5	0	6	200
C	1·5	3	3	327
D	22·5	3	3	468

through the nerve with the two interpolar distances were computed: with the short distance (outer edges of electrodes at least 2 mm. apart) the intensity was almost double that with the long distance (the values for the spread-free intermediate distance of 3 mm. were used for this calculation). This relative increase of ampèrage for electrotonus is in satisfactory agreement with that for excitation, and the observed decrease in polarizing voltage does not actually contradict the results obtained in stimulation.

B—*Alternating currents**

The relation between electrotonus and frequency is of particular theoretical significance, because the electrotonus does not involve some complications which occur in repetitive stimulation (see Holzer 1936; Schaefer and Göpfert 1936). Failure or diminution of reaction due to refractoriness or fatigue does not develop in subthreshold electrotonus. At frequencies of 2000–4000 hz. the electrotonus shows an uninterrupted train of even waves (observed with high vacuum cathode-ray equipment with linear time base, Marconi Works). Hence it is permissible to observe the electrotonus during a steady state at any frequency, thus avoiding the influence of phase at make of the current.

Since accommodation originates independently of excitation during the flow of a subliminal current, the continuous shift of the threshold condition may find some correspondence in a change of "polarizability". Now the results of excitation at low frequencies fit an equation (Hill, Katz and Solandt 1936) which embraces the time constant of accommodation in addition to that of excitation. An electrotonic equivalent of accommodation, therefore, would provide a physico-chemical basis for an appropriate descriptive term. The results, however, are not sufficient to establish this conception. Again, the electrotonic observations are compared with the excitatory results by means of an equation which represents closely the data obtained in liminal stimulation over the decisive frequency band.

1—*Time constants in A.C. electrotonus.*

We arrive at numerical values of k in two different ways. Hill (1936 *a*) has shown that the relation which connects the intensity I required for steady threshold stimulation (one response for each successive cathodic half-wave) with the frequency n is expressed by the equation

$$I/I_0 = \sqrt{\{(1+4\pi^2n^2k^2)(1+1/4\pi^2n^2\lambda^2)\}}, \tag{1}$$

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* In collaboration with O. Sager, Bucharest, Fellow of the Rockefeller Foundation.

where I_0 is the "true rheobase" for steady excitation by repetitive constant current pulses of sufficient duration, k and λ are the time constants of excitation and accommodation. There is an optimum frequency n_{op}, where I is a minimum. The strength of this optimum stimulus

$$I_{\text{op}} = I_0(1+k/\lambda).$$

k/λ ranges from 1/7·3 to 1/45 (Hill, Katz and Solandt 1936), and is on an average about 1/20 in a frog's sciatic under "normal" conditions (low calcium-ion content of saline, room temperature). Hence $I_{\text{op}}/I_0 \sim 1{\cdot}05$ (the same mean figure was obtained in determining I_{op} and I_0 directly in the same nerve). The ratio is sufficiently near to unity to allow a substitution in our calculation of I_0 by the measured value I_{op} without serious error. The value of I_{op} in electrotonus, of course, is relative to a definite height of catelectrotonic potential which amounted to 3·6 and 1·6 mV for intermediate distances of 0 and 2·5 mm. respectively. It was proved for various frequencies between 50 and 2400 hz. that the catelectrotonic potential was doubled when the polarizing current had exactly twofold strength. This result justifies the fixing of the electrotonic deflexion at an arbitrary subthreshold value.

Now if we assume that there is no accommodation in electrotonus or at least no noticeable accommodation above the optimum frequency, neglecting the λ-term and putting $I_0 = I_{\text{op}}$, we find that

$$k' = \sqrt{(I^2 - I_{\text{op}}^2)}/2\pi n I_{\text{op}}. \tag{2}$$

Even if there be an accommodation of the order observed in excitation, the error is small near the optimum frequency and already negligible at medium frequencies, if we write equation (1) in simplified form:

$$I^2/I_0^2 = 1 + 4\pi^2 n^2 k^2 \tag{3}$$

(Hill, Katz and Solandt 1936). Hence I^2 is a linear function of n^2 on the high side of the optimum frequency, i.e. if I^2 is plotted against n^2, the observed points are supposed to form a straight line which intercepts the I^2 axis at I_0^2. It is not necessary, therefore, to determine I_0 or I_{op} experimentally. From the diagram (see fig. 7) we read the value of n^2 where $I^2 = 2I_0^2$ and substitute this value in (3) obtaining equation

$$k = \sqrt{(1/4\pi^2 n^2)}. \tag{4}$$

While the graphical method (eq. 4) gives one value for k, the arithmetical method (eq. 2) gives values of k' for every frequency above the optimum frequency.

On an average of five experiments the graphical value for the electrotonic k at 0 mm. intermediate distance is 195 (177·3–220·8)μsec., which is close to the mean value derived from the voltage-capacity curve (185 μsec.). This concord indicates that (1) in the two cases the same quantity is being measured, and (2) its magnitude is not vitiated by experimental conditions. For a finite intermediate distance the time constant is considerably increased as in the measurements with condenser discharges; the absolute agreement, however, between the two methods is not so satisfactory, k at 2·5 mm. intermediate distance being of the order of 700 μsec. with sinusoidal currents while k at 3 mm. was 465μsec. with condenser discharges.

TABLE II—$I-n$ AND $E-n$ RELATION FOR CONSTANT CATELECTROTONIC POTENTIAL (3·6 mV, I.E. 18·3 % OF POLARIZING POTENTIAL AT 50 HZ.). INTERMEDIATE DISTANCE 0 MM.; INTERPOLAR DISTANCE 21 MM. SCIATIC NERVE OF HUNGARIAN FROG AT 22·5° C. BIRMINGHAM OSCILLATOR.

Frequency hz.	Polarizing potential 10^{-3} V	Polarizing current 10^{-7} A	k' 10^{-6} sec.	$I/\sqrt{n}$ 10^{-8}
50	19·7	2·22	—	3·14
100	21·3	2·36	574	2·36
200	22·0	2·52	428	1·78
400	25·0	3·10	388	1·55
750	33·8	3·89	306	1·42
1500	44·4	5·56	247	1·44
3000	64·0	8·74	202	1·59
4500	78·4	11·10	174	1·65
6000	94·0	13·50	159	1·74
8000	106·2	16·90	150	1·89
10000	119·6	19·85	141·5	1·99
12000	133·0	22·22	132	2·02

The conspicuous feature of the arithmetical values for k' is their dependence on frequency. With intermediate distance 0 mm. k' equals about 650 (549–727)μsec. at 100 hz. and falls with increase of frequency, first rapidly, then slowly, and reaches a value of about 190 (163–230)μsec. at 3000 hz. (mean of seven experiments, see fig. 6). Beyond this frequency the decline still continues with gradually diminishing steepness, k' attaining about 130 μsec. at 12,000 hz. (see Table II). This result agrees with the values calculated by Lullies (1930 *a*) from impedance measurements for the "internal" polarization capacity of nerve (sciatic of *R. temporaria*): between 64 and 4096 hz. the change of this capacity follows a course similar to that of k', decreasing by about two-thirds. This phenomenon is no peculiarity of living membranes; it also occurs in electrode systems (Lullies 1928; Fricke 1932); it may be partly due to the core-conductor structure (Blinks 1936).

2—Curve of the strength-frequency relation.

If the peak current I required for constant catelectrotonic deflexion (negative half-wave) is plotted against frequency n, the curve obtained rises with increase of frequency in a slightly S-shaped curvature from 100 to about 400 hz., continues as an almost straight line to 1500–2000 hz. and finally bends in a wide arc concave towards the abscissa (fig. 6). With decrease of frequency the curve falls from 100 to 50 hz. and rises again increasingly at 25 and 10 hz. (Table III). In nearly all the experiments where these frequencies were used the minimum was observed at 50 hz. (average temperature 24° C.); occasionally, however, the optimum frequency was shifted to 100 hz. which is the value in excitation at this temperature. When the optimum frequency was not determined, the intensity measured at 50 hz. was regarded as I_{op} for calculation of k'. When I was plotted against $\log_{10} n$, the symmetry of the two branches, predicted from Hill's equation and observed in excitation (Coppée 1934; Hill, Katz

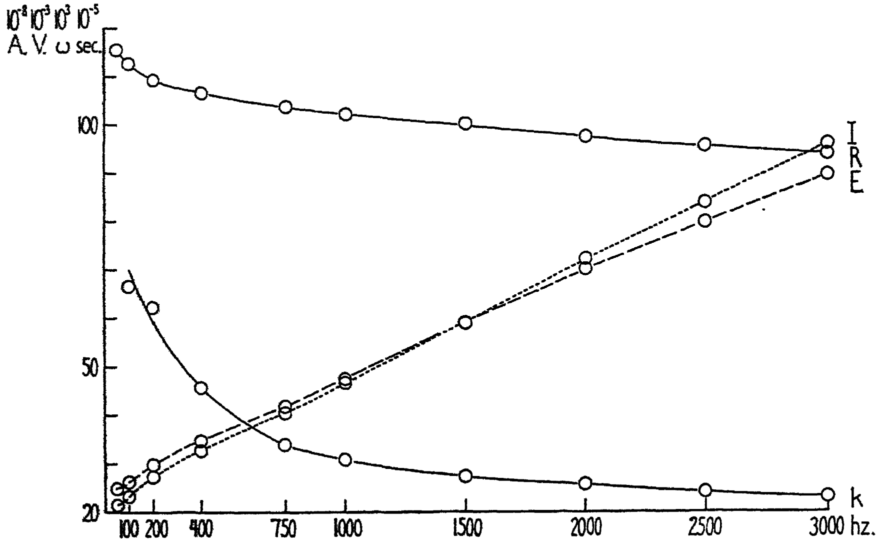

FIG. 6—$I-n$ and $E-n$ relations for constant catelectrotonus (3·6 mV), $n = 50$–3000 hz. (Harris oscillator). k' calculated from eq. (2). Impedance R (in ω) calculated from measured peak values of I and E. Sciatic nerve of Hungarian frog at room temperature (24·2° C.). Intermediate length 0 mm.; distances of leading-to and leading-off electrodes 21 and 33·5 mm. respectively.

and Solandt 1936), was not apparent. The rise of intensity seemed to be of different curvature above and below the optimum frequency (note that the onset of excitation precedes the catelectrotonic crest on the low-frequency side as the duration of the cycle increases; cf. p. 310). Because of the imperfection of the oscillator regarding setting, constancy and wave form in the range of lowest frequencies, the experimental points are not sufficiently close and exact for a definite statement.

TABLE III—$I-n$ RELATION FOR CONSTANT CATELECTROTONIC POTENTIAL (3·6 MV) AROUND OPTIMUM FREQUENCY. INTERMEDIATE DISTANCE 0 MM; INTERPOLAR DISTANCE 21 MM. SCIATIC NERVE OF HUNGARIAN FROG AT 25·1° C. BIRMINGHAM OSCILLATOR.

Frequency	10(?)	25	50 $= n_{op}$	100	200	400	500	hz.
Polarizing current	4·28	2·78	2·61 $= I_{op}$	2·87	3·14	3·74	4·09	10^{-7} A

The minimum of the electrotonic strength-frequency curve would be accidental if it could be reduced to an "abnormal" current distribution which should develop with "undercritical" interpolar length (Labes and Lullies 1932). If the interpolar distance equals the "critical" length required for "normal" current distribution at a certain frequency, an increasing fraction of the current is short-circuited by the envelope when the frequency is decreased. Hence the current applied must be intensified to compensate for this leakage. An interpolar distance of 21 mm., as used in these experiments, is probably sufficient for a "normal" current distribution down to the lowest frequencies employed. It is suggested, therefore, that the appearance of an optimum frequency in electrotonus reflects some peculiarities of polarization in nerve at low frequencies. Cole and Curtis (1936) observed an impedance element in frog's nerve with a maximum reactance at less than 100 cycles (see also Fricke and Curtis 1935) which, in accordance with our interpretation (Harris, Rosenberg and Sager 1935), they tentatively relate with an "adaptive mechanism". From similar measurements Labes and Lullies conclude that the diffusion seems to increase when, with prolongation of the cycle, the disturbance of concentration has passed through a layer which covers the outside of the nerve membrane and hinders diffusion (see Hill 1910). It is conceivable that such a process, which need not necessarily be affected by interpolar length, causes a loss of charge which in turn necessitates an increase of voltage applied in order to attain the same electrotonic potential. If the penetration of this diffusion barrier were dependent on temperature, the optimum frequency might shift with change of temperature—as observed in excitation.

Regarding the high-frequency end, it is shown that the electrotonic curve deviates from the straight line theoretically postulated for this region of the $I-n$ relation (fig. 6). Coincidence and difference within the experimental range above the optimum frequency are especially evident when I^2 is plotted against n^2. According to eq. (3) this relation is linear. This assumption actually seems to hold between 200–400 and 800–1600 hz. in one half of our experiments; it is this part of the series to which the values reported for k relate. For instance, in fig. 7 the points for 200, 350, 500, 750 and 1000 hz. form an almost perfect straight line which just misses the point for 1500 hz. (at 24° C.). In the other half of the experiments it is impossible to fit a straight line satisfactorily to the points observed; possibly in these cases the (quasi) linear region is so short that it is not defined by the frequency intervals chosen. In some experiments with an intermediate distance of 2·5 mm. the I^2-n^2 relation of electrotonus is also linear to the same upper limit.

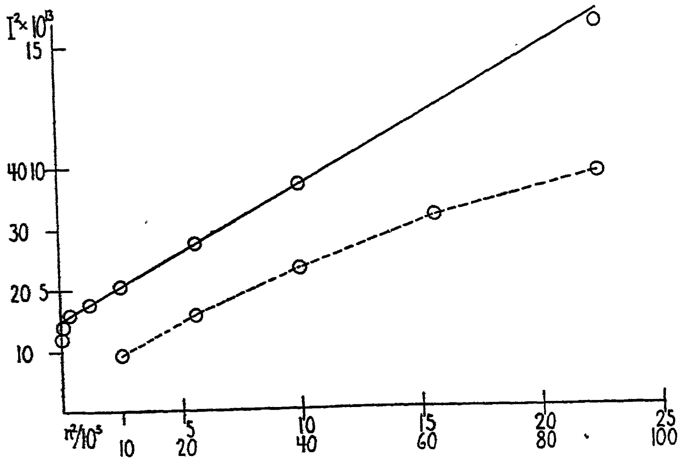

FIG. 7—I^2-n^2 relation for constant catelectrotonus (3·6 mV), $n = 50$–3000 hz. Sciatic nerve of Hungarian frog at 23·7° C. room temperature. Intermediate distance 0 mm. Distances of leading-to and leading-off electrodes 21 and 33·5 mm. respectively.

Upper curve (right ordinate and upper abscissa): 50–1500 hz. Lower curve (left ordinate and lower abscissa—scales a fourth of those of the upper curve): 1000–3000 hz. $k = 192$ μsec.

Above 800–1600 hz. the I^2-n^2 curve for electrotonus bends downwards, i.e. the observed points fall increasingly below the straight line as the frequency is raised. These data were obtained at a mean temperature of 24° C. In excitation the upper frequency limits for linearity are 470, 630 and 820 hz. on an average at 18, 22·5 and 28° C. respectively; exceptionally the linear relation was extended to 2000 hz. at high temperature; beyond the upper limit the excitation curve bent in a downward direction (Hill, Katz and Solandt 1936). Hence there is a general conformity between electrotonus and excitation curve above 200–400 hz. Below this frequency there is a striking divergence: the electrotonus points fall distinctly below the straight line while the excitation points rise slightly above it when they approach the optimum frequency. This rise in excitation corresponds with the increasing influence of the neglected accommodation factor λ, whereas the fall in electrotonus corresponds with the ignored increase of the time constant k'. In view of this discrepancy the identity of the accommodative processes in excitation and electrotonus seems problematical, and their exact relationship uncertain.

With intermediate distance 0 mm. the deviation from linearity at high frequencies is only in part due to increase of current spread with rise of frequency, since the curve takes a similar course with an intermediate distance of 2·5 mm., although the spread is enormously reduced (up to 800 hz. not noticeable at all). Hence the corresponding deviation of the excitation curve is probably caused by circumstances which affect stimulus and electrotonus in the same direction.

While the $I^2 - n^2$ relation formulated in eq. (3) is obeyed within a similar range of frequencies by both electrotonus and excitation, neither follows Nernst's square-root law. For excitation its invalidity was shown anew by Hill, Katz and Solandt contrary to Coppée's statement. Calculations of $I/\sqrt{n}$ for constant catelectrotonus proved that this expression is not constant anywhere over the whole explored band (see Table II). In some experiments the ratio appears to be approximately constant within a limited region. Actually, however, the values pass always through a minimum which sometimes is quite flat. Such cases suggest a pseudo-constancy of $I/\sqrt{n}$ comparable with that of V^2C in the voltage-capacity curve around the point of minimum energy.

With respect to linearity the $V^2 - n^2$ curve is inferior to the $I^2 - n^2$ curve. At optimum frequency and intermediate distance 0 mm. the catelectrotonic potential was, on an average of eight experiments, 15·9 (12·4–19·5) % of the peak potential applied which dropped almost entirely along the nerve between the points of contact of the polarizing electrodes.

From the similarity of catelectrotonus and excitation curves above 200–400 hz. we may infer, in accordance with analogous considerations by Schaefer (1936 *b*), that the negative half-wave is the stimulating phase of the cycle, i.e. the stimulus does not start when the positive half-wave begins to decline, as apparently Lullies (1930 *c*) and Coppée supposed, but departs from the abscissa at the onset of the negative phase, as presumed by Hill (1936 *a*).

IV—Discussion

In the foregoing section the results have been treated on an empirical basis with the sole assumption that the equations used for the comparison between electrotonus and excitation are correct representations of the data obtained in threshold stimulation. In Hill's (1936 *a*) theoretical definition the time constant k is the time factor of a "local potential" which decays exponentially when the applied current is withdrawn. In electrical terms this condition is realized by the conventional equivalent circuit for an irritable cell, consisting of a single static capacity c with ohmic parallel and series resistances r_1 and r_2 (see fig. 1) (Hermann 1905). This conception leads to expressions identical with Hill's equations (Rushton 1931; Schaefer 1936 *a*)—apart from the terms for accommodation which we can disregard in this connexion. The nerve fibre, however, is not represented by this simple scheme, but rather by a network embodying the electrical properties of a core-conductor which constitute the conditions for electrotonus. While the former involves an exponential rise and decline of the potential difference across the capacity when a rectangular pulse is applied to the ends of the circuit, the latter (fig. 1 without r_2) requires the electrotonic potential at the pole to change with make and break of a direct current according to the error integral of the square root of the time (Cremer 1932 *a*). The experimental conditions, however, do not fulfil the theoretical presumptions under which this function is derived; the curve of the electrotonic rise recorded at the pole, therefore, differs in some respects from the calculated curve and appears approximately exponential up to about 85 % of the final height. This seems to account for the fact that the equations valid for the elementary condenser circuit are applicable to the nerve, as far as the process at the pole is concerned.

Laterally from the pole, according to core-conductor theory and experiment in nerve, the electrotonic rise at make of a direct current is S-shaped and decreases in rate (and height) with increase of the intermediate distance. Although the conception related to the elementary scheme ignores these extrapolar phenomena, the experimental data fit the inadequate formulae to a surprising degree. This agreement, however, is perhaps fallacious: with a small intermediate distance (at room temperature) the S-curvature is scarcely perceptible, i.e. the initial (convex) part of the electrotonic rise is steep and short, the inflexion being near its origin, while the subsequent (concave) part, up to about 70% of the final height, approaches more or less an exponential course (see Bogue and Rosenberg 1934, fig. 4). The portion of the voltage-capacity curve, which corresponded with the initial part of the electrotonic rise, was probably missed, since the curve could not be explored below a given time limit (see § II). It seems possible, therefore, that the voltage-capacity curve for distant electrotonus, if it is continued towards the shortest discharge times with the necessary accuracy, will reveal, particularly at low temperature, a deviation from the asymptote for minimum quantity. This explanation, however, does not hold in either case, polar or extrapolar, for the last definitely non-exponential part of the catelectrotonic rise.

Finally, with regard to these discrepancies it is noteworthy that the core-conductor theory in its present form deals with a simplified single fibre and presumably has to be modified if it is applied to the actual conditions in a multifibre trunk. Because of the heterogeneity of fibre diameter, for instance, it is doubtful whether the capacity involved at medium frequencies is of the static or electrolytic type (Cole and Curtis 1936). At low frequencies polarization of some substrate introduces a new factor which the theory does not imply.

Independently of these theoretical difficulties the empirical consideration permits the following conclusions: The electrotonic voltage-capacity curve fits exactly the curve representing the results of threshold stimulation, and is shifted along the time axis in dependence on the interpolar distance as is the excitatory curve. The strength-frequency relation in electrotonus corresponds closely with that in excitation, not only in the range of medium frequencies where both satisfy Hill's equation (without accommodation term), but also in the range of higher frequencies where both deviate in the same direction from the theoretical I^2-n^2 line. The parallelism between electrotonus and excitation, therefore, is beyond doubt.

Although the time constants of excitation and electrotonus are of the same order, there remains a notable difference: the time constant of catelectrotonus at the pole is only about half that of excitation (at room temperature). If we compare maximum values of the catelectrotonic and minimum values of the excitatory time constant the difference is not significant, the former approaching the latter (250 μsec.). But in general k in excitation exceeds k in electrotonus substantially. This disparity necessitates an interpretation lest the whole conception is invalidated. We consider the following possibilities:

1—Schaefer (1936 *b*), who also observed great differences between half-time of catelectrotonic rise at the pole and chronaxie in threshold stimulation, suggested that in nerves of heterogeneous fibre diameters the time factors of electrotonus and excitation were not determined on the same fibre group. He supposed the establishment of electrotonus to depend on the time factor of the quickest fibres, whereas the most excitable fibres (related to rheobase) were not the most rapid (related to chronaxie). Without verification on single fibres or homogeneous fibre groups of different diameters the evidence for this conception seems insufficient.

2—Excitation and "membrane charge" as measured by electrotonus may not be confined to the same structure. According to Blair and Erlanger (1936) the delay of fibre action after a brief shock is due to a delayed attainment of critical voltage at the "responding mechanism": a large capacity is said to be rapidly charged and, after removal of the applied potential, slowly discharged through a resistance and small capacity (the excitable polarizable interface). From their experiments they calculate a time constant of 350 μsec. for the charge of this interface capacity which is in fact the excitatory k value. Since their figure is derived finally from excitation periods, the agreement is not demonstrative of the actual intermediate process.

Bishop (1928) supposed that in addition to the axon membrane the inexcitable connective tissue sheath was polarizable and influenced the course of charge and discharge of the irritable component. "The chronaxie of a bullfrog spinal nerve root from which the connective tissue sheath is absent is consistently one-half to two-thirds as great as for the corresponding nerve, and the utilization period is shorter, although the fibres stimulated in each case at threshold (presumably the largest fibres) should be the same." A part of the impedance at medium frequencies seems to be due to the presence of the sheath (Cole and Curtis 1936). These facts imply that the external sheath affects the time course of excitation but does not interfere with the development of electrotonus. The electrotonus, then, would measure the essential process at the axon membrane, while in excitation the time relations were distorted by the sheath.

3—The difference of the time constants might be caused by a sudden increase of capacity at excitation. Such an increase of polarization capacity (with simultaneous decrease of parallel resistance) is supposed to occur when the strength of the measuring sinusoidal current is raised until the nerve responds (Lullies 1930 *b*). Presumably this change is a consequence of the active excitatory event and does not develop before the onset of excitation, since the change subsides when the nerve begins to fail.

4—The time constant of electrotonus may concern the alteration of a cross-section immediately at the cathode, whereas the initiating process possibly has to affect a region of finite length in order to provoke excitation. Since the electrotonic "time constant" increases approximately proportionally with the intermediate length, at a distance of about 1·5 mm. k for electrotonus equals k for excitation. It has been shown that the time constants of excitation determined for the "minimum" electrical response at the stimulated point and after conduction are identical. It is suggested, therefore, that the development of excitation is not confined to one cross-section of the fibre, but requires the establishment of a potential gradient in the longitudinal direction, i.e. a critical catelectrotonus has to be generated over a certain length of nerve as a condition of "complete" excitation capable of propagation (Harris, Rosenberg and Sager 1935; Katz 1937; Rushton 1937).

This idea is supported by observations in Lillie's core-conductor model: "Activation requires more than a certain critical density of current, hence slightly scraping a passive wire in HNO_3 with glass may fail to activate the wire as a whole—i.e. fail to cause a transmitted effect—while a larger scrape activates" (Lillie 1929). Perhaps the internodal segment is the physiological unit of activation in myelinated axons (Erlanger and Blair 1934).

Apparently it is contradictory to the suggestion of "regional" excitation that the action potential begins before the polar catelectrotonic potential generated by a long rheobasic pulse has attained the full maximum. It is, however, conspicuous that the period between make and response determined in this way generally is shorter than the main utilization time measured for minimum response to rectangular shocks fashioned by a pendulum—i.e. probably in the electrotonic observations the strength was supraliminal because the action potential could not be sufficiently amplified under these circumstances. In addition, the catelectrotonic potential approaches its maximum asymptotically whereas the progression of accommodation prevents the nerve from responding after 3 msec. The same criticism applies to very slow condenser discharges in which it is difficult to ascertain exactly the start of the electrical response with respect to its position on the electrotonic crest.

Another essential difficulty for the stimulation hypothesis advanced is the different influence of temperature on the two processes. When the nerve is cooled both time constants are increased, but that for excitation considerably more than that for electrotonus. Between 4–6 and 19–23° C. the temperature coefficient Q_{10} of $1/k$ equals 1·8 in excitation (Hill 1936 *b*) and 1·2 in electrotonus between 2·5 and 20° C. (calculated from data published by Bogue and Rosenberg 1934). It seems a possible explanation for this discrepancy that a longer region of nerve is required for excitation at a lower temperature.

To summarize: the suggestion of "regional" excitation seems suitable to resolve the difference between the time constants, but it lacks conclusive evidence at this experimental state.

I am greatly indebted to Professor A. V. Hill for his inspiring interest in this work and to Professor D. T. Harris for his helpful technical advice.

SUMMARY

When an electric current is applied to a nerve a potential difference across the axon membrane is developed. It has previously been suggested that this potential is the internal stimulus, and that the attainment of a certain critical potential at the stimulating pole is the threshold condition for excitation. On the basis of the core-conductor theory it is assumed that this potential is measured when the electrotonic potential between the pole and a distant extrapolar point is determined. New experimental evidence with respect to this conception is submitted and compared with equations (Rushton, Hill) valid for excitation in nerve. The main results are:

The voltage-capacity relation for constant subliminal electrotonus fits closely the theoretical curve which represents the data for threshold stimulation with condenser discharges. Similarly the intensity-frequency relation agrees with theory and experiment of threshold stimulation by sinusoidal currents within a certain frequency range (200 and 1000 hz.) beyond which both electrotonic and excitatory curves diverge in the same direction from the theoretical line. With decrease of frequency below the optimum frequency, the current required for constant electrotonic potential shows an increase which suggests a physico-chemical factor in accommodation.

The time constants derived from observations on electrotonus at the cathode are only about half of those obtained in excitation. If, however, the time constants are determined for catelectrotonus at a short distance from the pole, they are comparable with those found in excitation. Hence

the hypothesis is advanced that the establishment of a gradient of critical potential along a finite length of nerve is the threshold condition for excitation. Other possibilities are considered.

A tentative explanation is offered for the empirical agreement of the results obtained in nerve, i.e. a core-conductor structure, with equations which theoretically apply to an inadequate equivalent circuit.

References

Bernstein, J. 1912 "Elektrobiologie", p. 146. Braunschweig.
Bishop, G. H. 1928 *Amer. J. Physiol.* **85**, 417.
Blair, E. A. and Erlanger, J. 1936 *Amer. J. Physiol.* **114**, 317.
Blinks, L. R. 1936 *J. Gen. Physiol.* **19**, 673.
Bogue, J. Y. and Rosenberg, H. 1934 *J. Physiol.* **82**, 353.
Bouckaert, J. P. and Katz, B. 1935 *J. Physiol.* **85**, 10*P*.
Cardot, H. and Laugier, H. 1914 *C.R. Soc. Biol., Paris*, **76**, 539.
Cole, K. S. and Curtis, H. J. 1936 Cold Spring Harbor Symposia on Quantitative Biology, **4**, 73.
Coppée, G. 1934 *Arch. int. Physiol.* **40**, 1.
Cremer, M. 1929 *Handb. norm. path. Physiol.* **9**, 244.
— 1932*a* *Handb. norm. path. Physiol.* **18**, 241.
— 1932*b* *Scientia* (3), **51**, 145.
Du Bois-Reymond, E. 1849 "Untersuchungen über thierische Elektricität", **2**, 333. Berlin.
Ebbecke, U. 1927 *Pflüg. Arch. ges. Physiol.* **216**, 448.
Erlanger, J. and Blair, E. A. 1934 *Amer. J. Physiol.* **110**, 287.
Fricke, H. 1932 *Phil. Mag.* (7), **14**, 310.
Fricke, H. and Curtis, H. J. 1935 *J. Gen. Physiol.* **18**, 821.
Harris, D. T. 1932 "Ultra-violet Radiology", p. 113. London.
Harris, D. T. and Rosenberg, H. 1935 *J. Physiol.* **84**, 11*P*.
Harris, D. T., Rosenberg, H. and Sager, O. 1935 *J. Physiol.* **86**, 4*P*.
Hermann, L. 1905 *Pflüg. Arch. ges. Physiol.* **109**, 95.
Hill, A. V. 1910 *J. Physiol.* **40**, 190.
— 1934 *J. Physiol.* **82**, 423.
— 1936*a* *Proc. Roy. Soc.* B, **119**, 305.
— 1936*b* *Proc. Roy. Soc.* B, **119**, 440.
Hill, A. V., Katz, B. and Solandt, D. Y. 1936 *Proc. Roy. Soc.* B, **121**, 74.
Holzer, W. 1936 *Pflüg. Arch. ges. Physiol.* **238**, 14.
Katz, B. 1937 *J. Physiol.* **90**, 6*P*.
Labes, R. and Lullies, H. 1932 *Pflüg. Arch. ges. Physiol.* **230**, 738.
Lapicque, L. 1907 *J. Physiol. Path. gén.* **9**, 620.
Lillie, R. 1929 *J. Gen. Physiol.* **13**, 1.
Lullies, H. 1928 *Pflüg. Arch. ges. Physiol.* **221**, 296.
— 1930*a* *Pflüg. Arch. ges. Physiol.* **225**, 69.
— 1930*b* *Pflüg. Arch. ges. Physiol.* **225**, 87.
— 1930*c* *Pflüg. Arch. ges. Physiol.* **225**, 98.
Rosenberg, H. 1931 *Ber. ges. Physiol.* **61**, 353.
— 1932 *XIV. Congr. int. Fisiol. Comunicaz.* p. 220.
— 1935 *J. Physiol.* **84**, 50.
— 1937 *Trans. Faraday Soc.* **33**, 1028.

Rushton, W. A. H. 1931 *J. Physiol.* **72**, 265.
— 1934 *J. Physiol.* **82**, 332.
— 1937 *J. Physiol.* **90**, 5*P*.
Schaefer, H. 1936*a* *Pflüg. Arch. ges. Physiol.* **237**, 722.
— 1936*b* *Pflüg. Arch. ges. Physiol.* **237**, 737.
Schaefer, H. and Göpfert, H. 1936 *Pflüg. Arch. ges. Physiol.* **238**, 404.
Schmitz, W. and Schaefer, H. 1933 *Pflüg. Arch. ges. Physiol.* **232**, 782.
— — 1934*a* *Pflüg. Arch. ges. Physiol.* **233**, 229.
— — 1934*b* *Pflüg. Arch. ges. Physiol.* **233**, 700.
Solandt, D. Y. 1936 *Proc. Roy. Soc.* B, **119**, 355.

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The External Mechanics of the Chromosomes

VI—The Behaviour of the Centromere at Meiosis

By Margaret Upcott

John Innes Horticultural Institution, Merton

(*Communicated by Sir Daniel Hall, F.R.S.—Received* 26 *June* 1937)

[Plate 14]

1—INTRODUCTION

In an attempt to analyse the forces governing the movements of chromosomes during division, variations from one cell to another and from one organism to another provide the only satisfactory method we know of determining the variables concerned, in short, of defining a "normal". By methods such as these it has been established that the same forces act in mitosis as in meiosis, and are merely co-ordinated differently. In meiosis the co-ordination is more complex and delicately balanced than in mitosis. It is therefore more easily disturbed, and its variations are more frequent and of many different kinds. Moreover, even if lethal to their products, they are not lethal to the organism, as even slight variations of mitosis usually are; hence they are more easily studied. Each of these kinds of variation may give a clue to some problem which in the "normal" remained obscure or unnoticed. Each is a natural experiment which, while not usually disturbing the process so far as to cause death as man-made experiments often do, leads us by comparison to a further understanding of the normal.

The variations may be of several kinds. They may be determined by the possession of an abnormal genotype (Darlington and Thomas 1937), or by structural or numerical changes in the chromosomes. They will affect all the cells undergoing meiosis although perhaps in varying degrees. A further set of variations consists in temporary and environmental abnormalities affecting single cells or groups of neighbouring cells.

The present study includes a few such isolated examples, but is concerned mainly with the deviations from the normal which the possession of an extra set of chromosomes brings about, and with the conditions under which these extra chromosomes divide. The material from which the comparisons were made consists of meiosis in the following diploid and triploid species and varieties of *Tulipa*:*

T. Orphanidea	$2x = 24$
T. praecox	$3x = 36$
T. "Gesneriana" vars.:	
Mrs. Moon	$2x = 24$
Philippe de Commines	$2x = 24$
Inglescombe Yellow	$3x = 36$
Massenet	$3x = 36$
Pink Beauty	$3x = 36$
Keizerskroon	$3x = 36$.

2—THE ACTION OF THE SPINDLE ON THE CHROMOSOMES

In all the plants studied, dicentric chromatids are formed at meiosis as a result of crossing-over between relatively inverted segments of chromosomes pairing during prophase (Upcott 1937; Darlington 1937, p. 575). These chromatids form bridges at the first and second anaphases. Owing to their special structure, the movement of dicentric chromatids at these stages is likely to throw light on the forces determining the movement of all chromosomes at this stage. Their behaviour is mainly of two kinds, according to whether the bridges are short or long.

As the centromeres (Darlington 1936) separate during anaphase the chromatid joining them is pulled out and becomes thinner. The effect of this stretching varies according to the length of the bridge. There is even during metaphase some tension in the chromatids joining the two centromeres in an ordinary bivalent where the first chiasma is close to them. At anaphase this tension increases. If the bridge is short, extreme stretching begins early and is either uniform from centromere to centromere, or greatest in the middle (Upcott 1937, figs. 18 and 19) where it usually breaks before telophase.

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* The slides were prepared by smearing the pollen mother cells, fixing in medium Flemming and staining with gentian violet according to Newton's method.

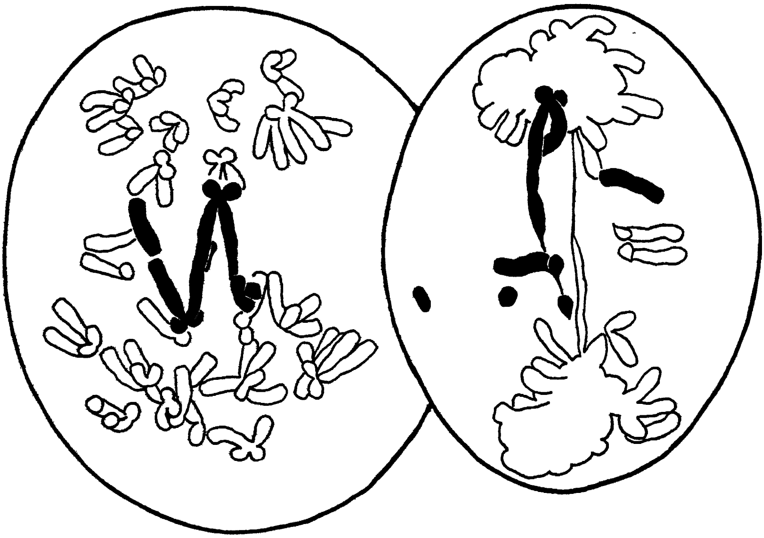


FIG. 1 FIG. 2

FIG. 1—Trivalent bridge at the early anaphase of the first division in "Inglescombe Yellow". There has been crossing-over in a duplication to give the short arm attached to the right-hand centromere, which should have a long arm as the left-hand centromere has. There has also been crossing-over between the three homologues in an inverted segment. The two unequal fragments are visible (× 2000).

FIG. 2—Late anaphase in the same plant. There are two bridges which have been uncoiled along part of their length by the stretching of the spindle. In the left-hand bridge (shown in black), which is asymmetrically placed, the free short arms have been pulled apart at one centromere, but not at the other (× 2000).

If the bridge is long, this extreme stretching does not begin until late anaphase, and when it begins it affects the chromatid unevenly, and particularly near the centromeres (figs. 2 and 3*b*). These parts may be pulled out to a fine thread while the central region still remains closely coiled.

In certain triploids, the two free sister arms may become separated from one another at the centromere (figs. 3, *c*, *d* and *e*; Upcott 1937, fig. 19*d*), usually, for an unknown reason, when the bridge is in the short arm. This phenomenon is particularly noticeable in bridges which are placed asymmetrically with regard to the equator (fig. 2). In these cases we find that the end which is included in the daughter nucleus is normal, while the other which lags on the spindle is stretched.

These differences of behaviour between short and long bridges and between early and late anaphase bear out the view, put forward by Belar (1929) and extended by Darlington (1936), that the anaphase separation is determined by two agents, first by the mutual repulsion of the centromeres and later by the stretching of the central region of the spindle, which completes the process by pushing the chromosomes farther towards the poles.

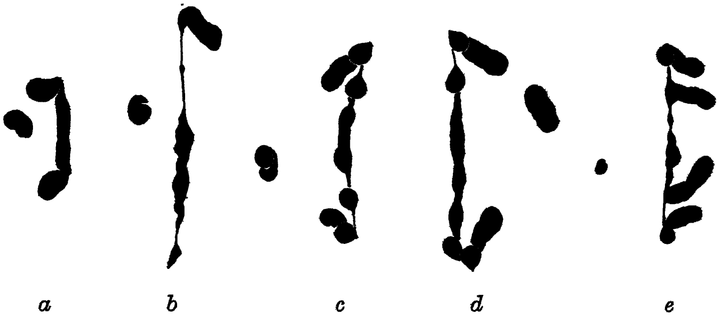


FIG. 3—Further examples of the stretching of bridges, and of the pulling apart of free arms (×2000). *a* and *b*, *T. Clusiana* (5*x*) early and late anaphase; *c*, "Pink Beauty" (3*x*); *d*, "Massenet" (3*x*); *e*, "Pink Beauty". Here the bridge is in the short arm.

The behaviour of dicentric chromosomes provides us with further evidence of the second process and enables us to distinguish it from the first.

At early anaphase in dicentric and in freely separating chromosomes the centromere leads, hence the name "Leitkörperchen" given to it by its discoverer (Metzner 1894). Further, when short bridges stretch at this stage they stretch evenly. We may assume from these two observations that the centromere repulsions are governing the process at this stage, and we may even estimate the limits over which this repulsion is effective.

At late anaphase, on the other hand, we have three observations which show that the movement of the spindle is carrying on what the centromere repulsions began. First, in bridges, the centromere no longer leads, although it still does so in freely separating chromosomes (figs. 13 and 15). This must be because freely separating chromosomes can move as a whole, so that their shape is not altered by the pushing of the spindle, while bridge chromosomes can only move in the free part. Secondly, long bridges stretch unevenly at this stage and the stretching occurs near the centromeres. Thirdly, this stretching may lead to the separation of sister arms at the centromere, particularly in asymmetrically placed bridges.

The last two observations show us not only that the spindle expands, but actually where it expands. There can be no movement in the equatorial regions where the future cell wall will be laid down, since chromatids lying in this region remain closely coiled. All the movement must take place in two zones between this and the poles, for it is here that the uncoiling occurs. This supposition is confirmed by the behaviour of bridges in which only one end lies in the expanding region (fig. 2). It must also be supposed that the region immediately adjacent to the centromere is more susceptible to uncoiling, since the central region of this bridge which is lying in the second expanding zone of the spindle has not uncoiled. The second bridge which this cell contains must also have been asymmetrical, for it has uncoiled here but not at the other end. The fact that the strain is not transmitted equally across the bridge, but remains localized, suggests that there is considerable friction between the chromosomes and the spindle material.

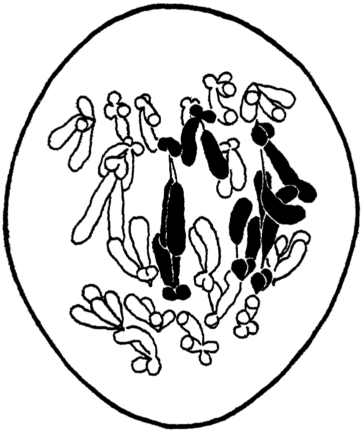


FIG. 4—Anaphase of the first division in *T. Orphanidea* (2x). The chromosomes of three bivalents show temporary constrictions next to the centromere, presumably comparable to those found in bridges (× 2000).

This separation of the free sister arms at the centromere differs from the simple cases of stretching in that one of the free chromatids is affected as well as the bridge chromatid. That free sister arms can separate at the point where we assume they are each attached to the centromere is unexpected. It provides further evidence of the existence of a differentiated surface or pellicle surrounding the coiled thread and joining parts of it which would otherwise be separate, in this case, joining the bridge to one of the opposite arms (Darlington 1932, p. 290). Abnormally dividing univalents, as we shall see, support this view.

Uncoiling of the chromosome thread in this way by the expansion of the spindle is usually confined to bridges, since here there is exceptional resistance to movement. But occasionally interlocking of free, that is to say, of monocentric, chromatids of a bivalent presumably following the formation of comparately related chiasmata sets up a similar resistance. The expansion of the spindle then produces a similar result. In one exceptional cell (fig. 4), three bivalents each have two constrictions next to the centromere; in two of them the constrictions lie in corresponding chromatids of opposite chromosomes, while in the third, both constrictions are in one chromosome, its homologue being without them. This difference is perhaps attributable to different positions on the spindle.

The constrictions which I have described here differ from nucleolar or secondary constrictions in their position, in their transitory nature and in the stage at which they are visible. They are not confined to *Tulipa* but have been observed also in *Lilium* (Richardson 1936, figs. 40–43, 52; Ribbands 1937, figs. 49–58) and in *Fritillaria* (Bennett 1937) at the same stage, and in *Tradescantia* at anaphase of the pollen-grain division (Upcott, unpublished).

3—THE BEHAVIOUR OF UNIVALENTS

a—Types of univalent

At the first anaphase of meiosis in a triploid, a variable number of univalents lag upon the plate as the other chromosomes pass to the poles. It has been shown (Darlington 1929) that these laggards are of two kinds. They may be true univalents, that is, those which formed no chiasmata with either of their partners at prophase and which therefore were unpaired at metaphase; or they may be members of trivalents or other multiple associations left upon the plate after the separation of their partners.

Hitherto it has been possible to distinguish between these kinds of univalents in two ways, statistically by comparison of metaphase and anaphase frequencies or by the formation of univalent bridges which occur only rarely and in exceptional circumstances (Upcott 1937). But they may be distinguished also by the position of the chromatid arms. Darlington (1929) has shown that if chiasmata have been formed in one arm only of a bivalent or trivalent, the chromatids of the arm without chiasmata remain together at anaphase instead of separating widely as they do in the arm with chiasmata. We find the same distinction in univalents. In some the chromatids lie together, or parallel but slightly separated (figs. 6*a*, 8). We may then say that these univalents have been unpaired at metaphase. In others the chromatids spread out widely on either side of the centromere (figs. 6*b*, 12 and 13), in which case they must have been parts of trivalents. At early anaphase it is often possible to recognize the two other members, while the ends of previously associated chromatids remain touching (fig. 5).

This difference in position of the arms is presumably due to a difference in the relationship of the coils of the chromatids. At metaphase, sister chromatids generally share the same major coil, but at anaphase they slip free of one another, a process which must be initiated by a lapsing of specific attractions and hastened by the separation of chromatids having one or more changes of association, occasioned by chiasmata. In a univalent this slipping free is conditioned only by the lapsing of attractions. It does not therefore proceed so far, so that the chromatids are closer together even while the centromere is dividing.

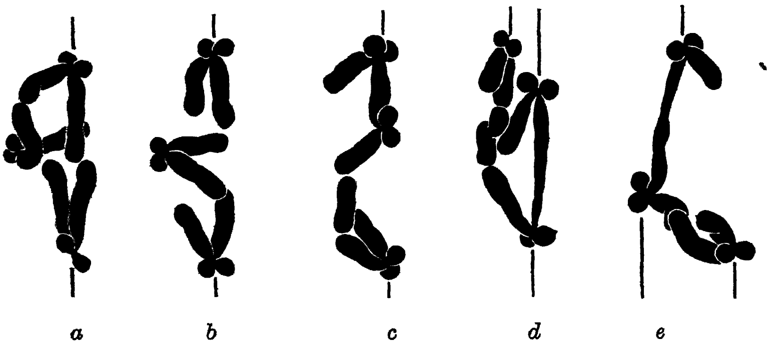
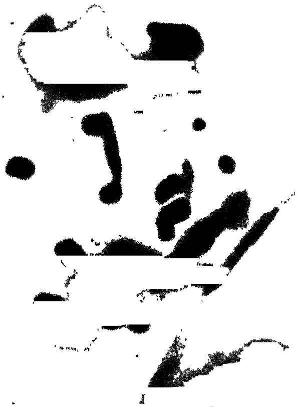


FIG. 5—Dividing trivalents from anaphases in "Inglescombe Yellow" (×2000). *a* and *b*, Linear or indifferent; *c*, linear; *d* and *e*, convergent or indifferent. The last three configurations contain a bridge between two members.

We have now distinguished two kinds of univalents at the first anaphase of meiosis, "true" univalents which have not formed chiasmata (fig. 6*a*) and lagging members of trivalents or "open" univalents, which have (fig. 6*b*). But there is a third kind which can occur only in special circumstances, that is, where there is crossing-over in inversions. This is the "loop" univalent (figs. 6*c* and 15; Pl. 14, *c* and *d*), which I have already described (1937). It arises as a result of a chiasma in the inversion as well as a proximal one disparate to it. Univalents of this type will give rise to univalent bridges (fig. 7) if the centromere divides at the first instead of at the second division.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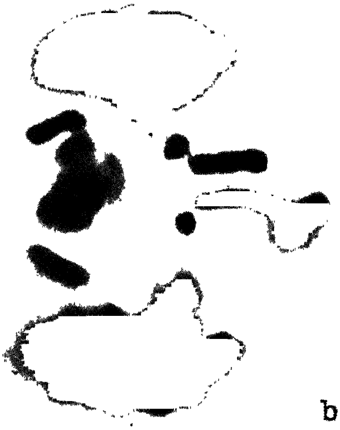
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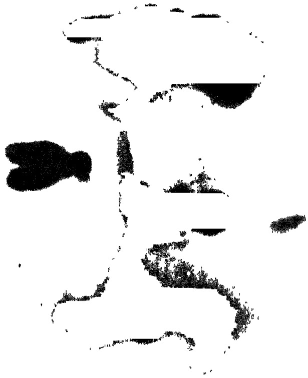
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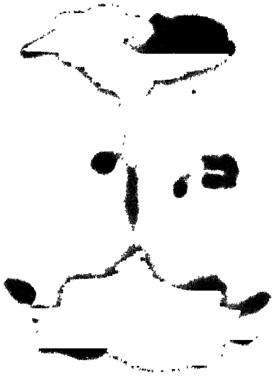
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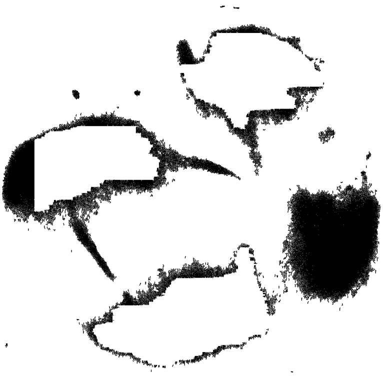
e

f

Trivalents, however, do not always give rise to lagging chromosomes either of the "open" or the "loop" kind. Whether they do so or not depends upon their orientation at metaphase, and this in turn depends upon the number and position of their chiasmata. Darlington (1936, 1937, p. 575) recognizes three types of co-orientation in trivalents, namely, convergent, linear and indifferent, according to the relationships of the three centromeres on the spindle. It is clear that when the arrangement is convergent, lagging chromosomes will not occur but may do so when it is linear or indifferent (fig. 5*d* and *e*). After inversion crossing-over, these latter arrangements may lead to disjunction or non-disjunction of the dicentric chromatid so formed, according to whether or not its two centromeres pass to opposite poles (fig. 5*c*–*e*).

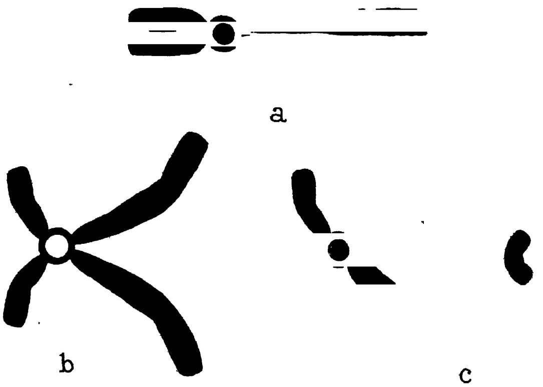

FIG. 6—Diagram showing three types of univalent distinguishable at the first anaphase of meiosis. *a*, true univalent, that is, one which has not been part of a trivalent (cf. fig. 8); *b*, lagging member of a trivalent, which will behave as a univalent after being left on the plate (cf. fig. 5); *c*, closed loop univalent, derived from crossing-over in an inversion and proximal to it. This will give a univalent bridge when its centromere divides (cf. Pl. 14, *c*).

b—*Division of univalents*

Univalents which lie on the plate after the main body of chromosomes pass to the poles may suffer one of several fates. They may either divide or remain undivided, and divided or undivided they may be included in one of the daughter nuclei or remain lagging on the plate. Presumably their fate is determined by their position. All the triploids I have examined have varying numbers of univalents, a varying proportion of which divide at the first division. They are most numerous in "Inglescombe Yellow" and

"Massenet", which, however, differ from one another in behaviour at the second division. To elucidate this I have studied them in some detail, but I propose to discuss only the qualitative data here, and to give the quantitative data elsewhere in relation to genetic structure.

At the first division the univalents of "Massenet" do not differ from the normal, that is to say, the centromeres of those which divide do so in a longitudinal plane, so that two daughter univalents are formed, each with two unlike arms, in this case one long and one short (fig. 18). At the first division of "Inglescombe Yellow", however, the univalents, or more correctly their centromeres, may divide in almost any plane, depending presumably upon their orientation when their polarity first developed. I will now describe these abnormalities in detail.

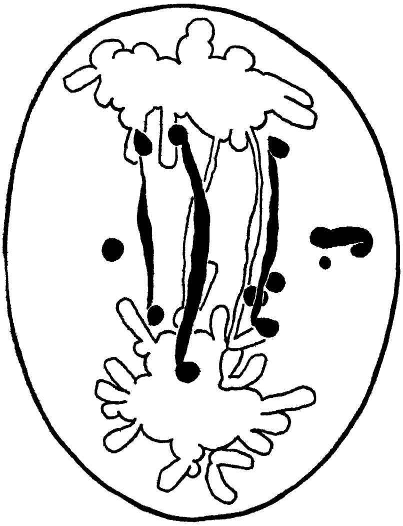

FIG. 7—Anaphase of the first division in "Massenet" (× 2000). The cell contains five bridges, of which three are univalent bridges and must have been closed loop univalents at an earlier stage. There are five corresponding fragments.

The simplest deviation from the normal is that where, after the centromere has divided in the usual longitudinal plane, a constriction appears next to the centromere, in one or both daughter univalents (figs. 9, 10 and 11, left-hand univalent, and 18 (1)). This constriction is presumably of the same kind as those which appear in bridges and occasionally in free chromosomes, that is to say, it must be due to the stretching of the spindle. At a later stage the constriction may break, so that daughter univalents occur consisting of single arms, the second arm without a centromere lying nearby (figs. 10*c* and *d*, 16). Further, univalents which have divided normally are often connected by a fine thread (figs. 9, 10*b*). This peculiarity again suggests the presence of a pellicle covering the chromosome and connecting parts of it not joined by the continuous chromosome thread.

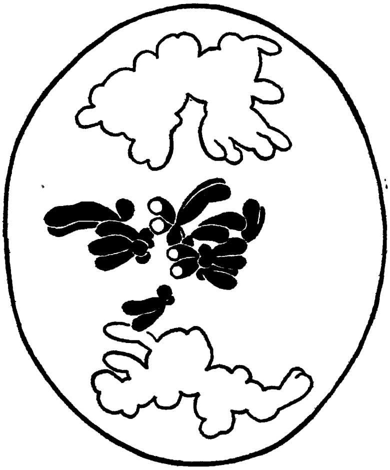

FIG. 8

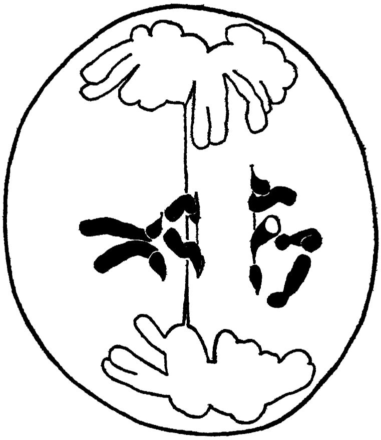

FIG. 9

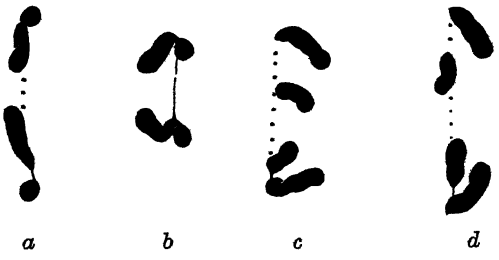

FIG. 10

FIG. 8—First anaphase in "Inglescombe Yellow" in which eleven univalents are forming an accessory plate (× 2000).

FIG. 9—Abnormal division of univalents at the first anaphase in "Inglescombe Yellow". A thread connects the daughter univalents in two cases (× 2000).

FIG. 10—Further examples of abnormal division (× 2000). *a*, a constriction has formed at the centromere in one daughter univalent; *b*, daughter univalents joined by a thread; c, and *d*, the two arms of one daughter univalent have separated, while in the other a constriction has formed (cf. fig. 18 (1)).

The second type of division is rare. It deviates even farther from the normal. Here the centromeres must have divided transversely to the two arms, for we find daughter univalents consisting of a pair of sister arms separating from others consisting of the complementary pair (figs. 11, 18 (2)). In effect the chromosome has fragmented at the centromere.

The third abnormality is more common and consists in the separation of three arms attached to one daughter centromere and one to the other (figs. 12, 13, 14 and 18 (3)). This may occur in open univalents and also in loop univalents (fig. 15; Pl. 14, *d*). Often the centromeres themselves are visible and appear as small granules attached asymmetrically to the dividing chromosome. The connecting thread mentioned earlier again appears, usually connecting sister arms. The stretching of the spindle seems particularly to affect this type of separation, leading to the constriction and breaking of one of the arms. Perhaps movement is restricted on account of the greater bulk of the three arms attached to one daughter centromere so that the chromosome remains longer in the part of the spindle that stretches.

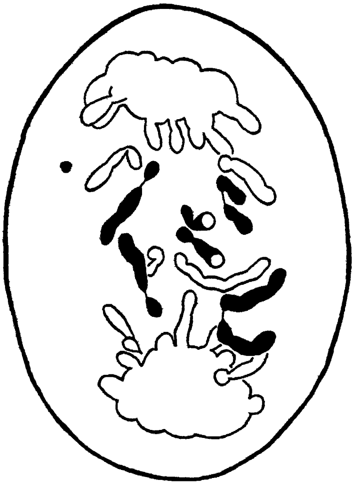

Fig. 11—First anaphase in "Inglescombe Yellow" containing several dividing univalents. The one on the right has divided so that the two short arms pass to one pole and the two long arms to the other (cf. fig. 18 (2)).

In the fourth abnormality the division is apparently normal in relation to the two arms, but at an early stage breakage occurs next to the centromeres, and the two centric arms continue their passage to the poles leaving the other two arms acentric and passive on the plate (figs. 16, 17 and 18 (4)). This may be a modification of the first type of abnormality. It differs from it in the earlier breakage of the arms, i.e. before the separation has proceeded far, and also in that the two arms left without a centromere are joined by a thread (fig. 17). Occasionally both pairs of arms are joined in this way (fig. 17*d*).

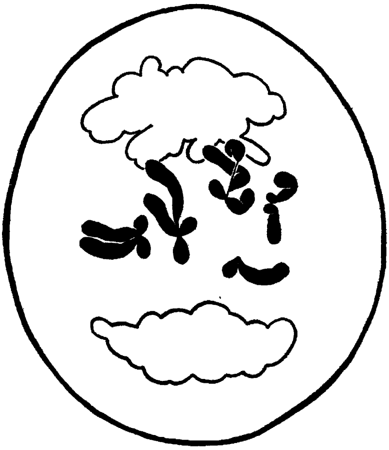

FIG. 12

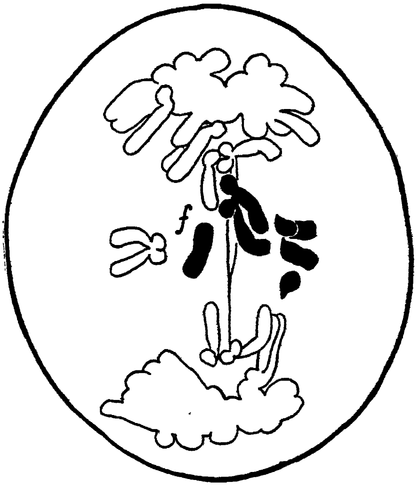

FIG. 13

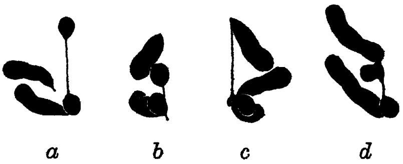

FIG. 14

FIGS. 12, 13, 14—Univalents at the first anaphase of "Inglescombe Yellow" dividing so that three arms pass to one pole and one to the other. In fig. 14 (*d*) the centromeres are visible (× 2000) (cf. fig. 18 (3)).

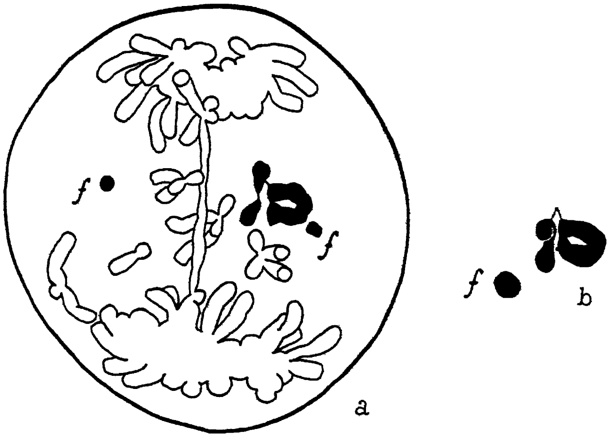

FIG. 15*a* and *b*—Two closed loop chromatids in "Inglescombe Yellow". Both are dividing so that three chromatids are attached to one centromere and one to the other (cf. Pl. 14, *c* and *d*). (× 2000.)

From these abnormalities we gain a new insight into the properties of the centromere. First it would appear that when the centromere divides it always divides into two. It must presumably have become bipolar before it can divide. In this respect therefore its division is of the same kind as the division of the chromomeres. These, however, divide at a moment when no spindle exists to determine their polarity, and there is some evidence that the plane of division may differ in successive chromomeres. Oblique or transverse divisions such as I have described here in the centromere have not, however, been either observed or inferred in chromomeres.

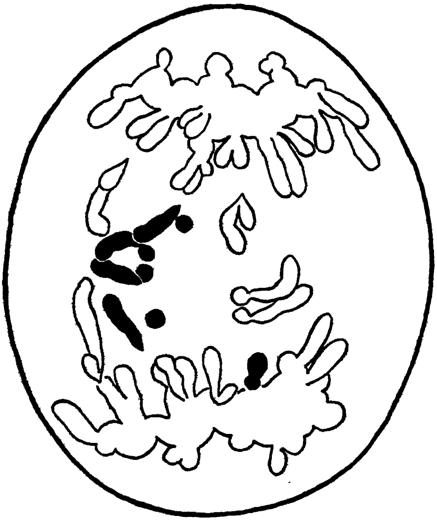

FIG. 16

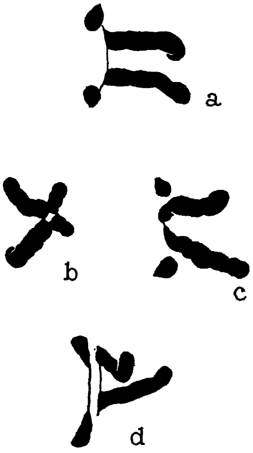

FIG. 17

FIGS. 16 and 17—Univalents at anaphase in "Inglescombe Yellow" (× 2000). Fig. 16, entire cell in which opposite arms of two univalents have broken at the centromeres. Fig. 17*a* and *b*, early stages (cf. Pl. 14, *b*); *c* and *d*, later stages of the same type of abnormality (cf. fig. 18 (4)).

Secondly, we see that the polarity which we assume the centromere to acquire before division is not predetermined. It must be determined in a way similar to that in which the co-orientation of two or more centromeres is determined at an earlier stage. If the bipolarity of the centromere is determined so that it will divide in the plane of the equator, we must assume that these abnormal univalents were abnormally orientated when the polarity was first determined. But it is evident that the reaction between spindle and centromere differs from one organism to another, since the four types of abnormality just described occur only in "Inglescombe Yellow" although other comparable abnormalities occur at the second division in other plants.

Thirdly, although we see that the centromere may divide asymmetrically with respect to the chromosome, i.e. so as to separate an unequal number of chromatid arms, yet there is often a certain symmetry in these abnormalities. When one chromosome arm breaks away from the daughter centromere in each daughter univalent, leaving the centromeres attached to one arm only, we find it is always the corresponding or sister arms which remain attached (figs. 10, 16 and 17). Further, two arms which are normally connected through the centromere never remain connected without it, but always separate leaving an attaching thread which becomes

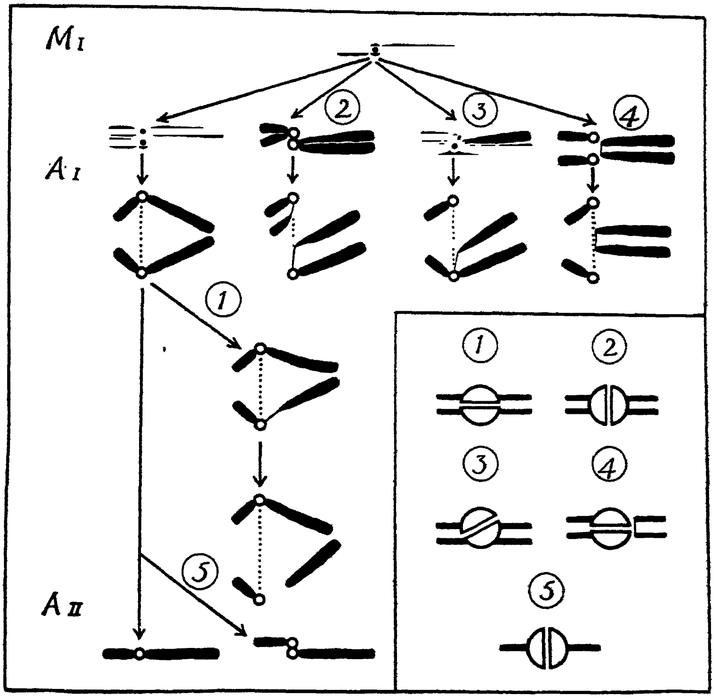

FIG. 18—Diagram to show the various ways in which the centromere of a univalent may divide. The first column shows the normal behaviour. This may be modified so that the long and the short arms of one or both daughter univalents break apart (1). The univalent may also divide transversely (2) or obliquely (3) or in a manner which separates one pair of arms entirely (4). Further a daughter univalent which has already divided normally at the first division may divide transversely at the second (5). Inset: behaviour of the centromere such as would give the observed abnormalities. $M_{\rm I}$ = first metaphase, $A_{\rm I}$ and $A_{\rm II}$ = first and second anaphase.

finer and finer until it breaks. Thus the pellicle inferred earlier is not an effective connexion without the centromere.

c—Univalents at the second division

We have seen that the two triploids "Inglescombe Yellow" and "Massenet" resemble one another in having a number of univalents which lag and divide at the first anaphase, but differ in the method of that division, "Massenet" being normal in this respect and "Inglescombe Yellow" abnormal. When we come to study the second division we find they differ in further ways.

Let us first consider what happens in "Inglescombe Yellow". Here we find that the chromosomes at second metaphase are relatively long and thin, almost indeed of mitotic length (fig. 19). Owing perhaps to this greater length and the lower surface charge associated with their reduced contraction they are greatly distorted by fixation; in fact their reaction at metaphase is that proper to prophase. It is possible to distinguish daughter bivalents from daughter univalents only occasionally. A few can be recognized in favourable cells (fig. 19) when the daughter univalents are seen to lie off the plate towards the poles.

At anaphase (figs. 21, 22) the fixation improves. One or two daughter chromosomes may occasionally lie passively on the equator. These must be daughter univalents which have divided at the previous division. But the number of these lagging chromosomes is very much less than the number we saw dividing at first anaphase, even if we disregard those which divided abnormally. Presumably then the remainder are carried to the poles along with the dividing chromosomes.

At second metaphase in "Massenet", on the other hand, the chromosomes are short and contracted, hardly longer than they are at the first division (fig. 20). They withstand the action of the fixative, so that daughter bivalents and daughter univalents can be readily distinguished by their structure. It now becomes clear that daughter univalents at second metaphase behave in the same way as univalents at first metaphase, that is, they lie off the plate. Occasionally one or more may be included on the plate along with the whole chromosomes. The daughter univalents in both nuclei can also be seen to lie towards the dividing wall, as though they had not moved to any appreciable extent since the previous anaphase.

At anaphase, as the daughter bivalents divide and move to the poles, the daughter univalents congress and form a secondary plate of their own (figs. 23, 24). This congression may be taken to indicate a development of polarity in the centromeres, such as precedes and determines the division

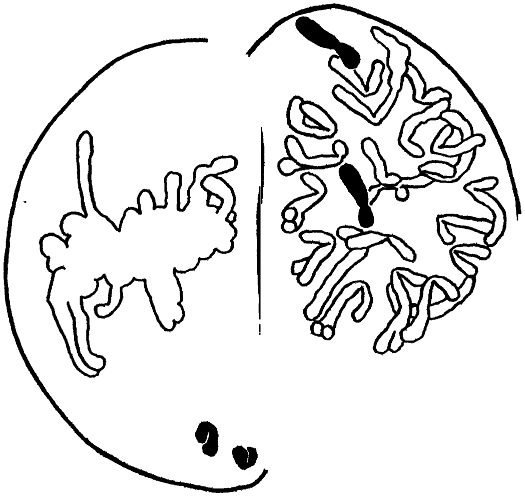

Fig. 19

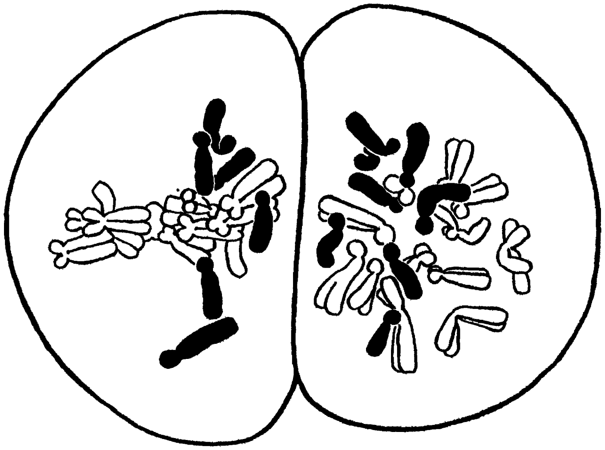

Fig. 20

Figs. 19, 20—Metaphase of the second division in "Inglescombe Yellow" (fig. 19) and "Massenet" (fig. 20), showing the difference in degree of contraction. Daughter univalents lie off the plate and are shown black. (×2000.)

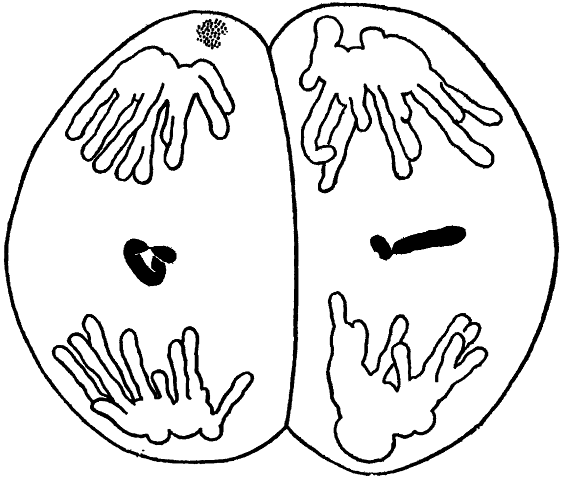

Fig. 21

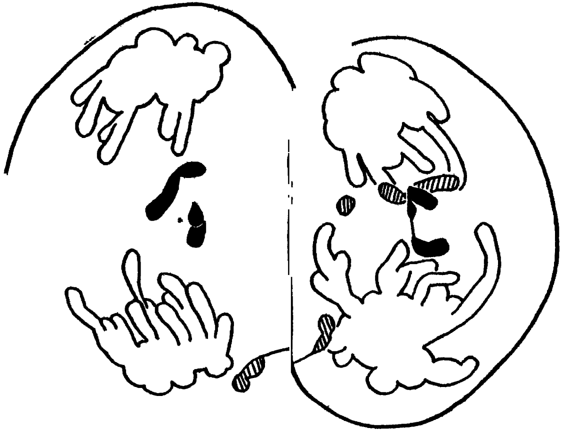

Fig. 22

Figs. 21, 22—Second anaphases in "Inglescombe Yellow", showing the relatively long thin chromosomes and the few lagging daughter univalents. Fig. 21. This cell has a short first-division bridge which has been cut by the wall and slightly shifted in the process. The right-hand daughter cell contains a short second-division bridge. (× 2000.)

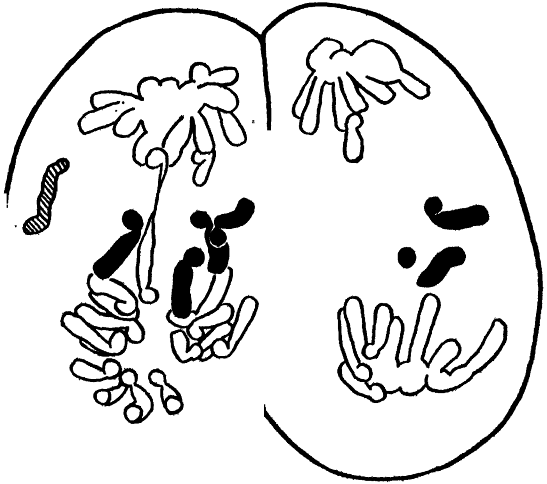

FIG. 23

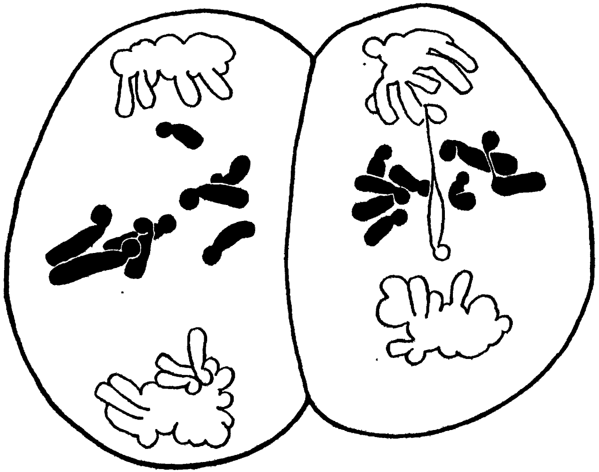

FIG. 24

FIGS. 23, 24—Second anaphases in "Massenet" (×2000). The chromosomes are shorter and thicker than in "Inglescombe Yellow" and the daughter univalents form an accessory plate. Some of them are at various stages of transverse division.

of univalents at the first division and indeed of ordinary mitotic chromosomes. But here the chromosomes themselves have not divided, and we then find the polarization of the centromere takes place in the only direction in which it can lead to the separation of the chromosome into two parts, namely, transversely, breaking the chromosome into two fragments (figs. 18 (5), 23 and 24). The centric constriction at the earliest stage of this division lies in the axis of the spindle. It then becomes elongated and eventually breaks. This happens at the end of anaphase after most of the spindle stretching has taken place, and just before the spindle lapses at telophase. It is not therefore surprising that these doubly divided univalents should rarely be included in the nucleus. Their division in this way is not uncommon, but the divided arms never move very far and are consequently lost in the cytoplasm.

We see therefore that the course of the second division in "Inglescombe Yellow" differs from that in "Massenet" in three respects: first, in the reaction of the metaphase chromosomes to the fixative; secondly, in their degree of contraction; and thirdly, in the behaviour of the daughter univalents at anaphase. These differences are such as may be attributed to differences of timing, but with our present knowledge we cannot define them more exactly since we know little of the relationship of the coiling systems at the first and second divisions.

The comparisons, however, show, first, that lagging is a lagging in congression as well as in division, and secondly, that it is not due to inactivity, as the word suggests, but to a different and later time cycle. This has already been made clear by Kihara (1931) at the first division. It is now equally clear at the second.

d—Double division of univalents

Daughter univalents, fragmenting transversely or lying in the axis of the spindle with their centric constriction much elongated, have been observed and figured in various other organisms, particularly oats (Nishiyama 1931; Katayama 1935; Philp, unpublished) and also in an aberrant strain of *Pisum* in which univalents are common (Koller, unpublished). This behaviour has been explained by Darlington (1937) as being due to the transverse division of the centromere. The present observations of abnormally dividing centromeres at the first division support this view. It is probable that the phenomenon occurs in other organisms with univalents in which some divide at the first division and lag at the second. Indeed, it seems to offer an explanation of certain cases of double division of univalents which have been reported, usually in organisms with rather small chromosomes

(Darlington 1937, Table 65 (B)). That double division occurs is inferred from chromosome counts at the second anaphase. But where the chromosomes are small, as in *Rosa* (Erlanson 1929), *Prunus avium nana* (Darlington 1930) and *Raphanus-Brassica* (Karpechenko 1927), it is difficult or impossible to distinguish the two arms either at the first or the second anaphase of meiosis. Hence it would not be possible from anaphase counts to say whether the extra univalents had divided transversely or longitudinally, by the entire chromosome thread dividing a second time, as in mitosis.

The two cases of doubly divided univalents which this explanation will not cover are those found in *Pygaera* and *Ribes* hybrids (Federley 1931; Meurman 1928). In the first we have genetical evidence that the chromosome thread itself divides a second time. In the second, Meurman finds occasional pollen mother cells having sixteen univalents at the first metaphase and others having sixteen presumably daughter univalents in each second metaphase plate, giving four unreduced daughter nuclei. It is, however, possible that these pollen mother cells, which are distinguished from the others by being larger and by having larger nuclei, may be tetraploid and produced by earlier somatic doubling, such as often occurs in the anthers of hybrids. The bodies in the cell would then be bivalents, their slightly smaller appearance being due to an increased chiasma frequency brought about by the doubling.

In both these hybrids there is often complete failure of pairing at the meiotic prophase. It is therefore probable, as Darlington (1930) points out, that the double division of the chromosomes is in some way determined at pachytene by the failure of pairing, perhaps by the earlier division of the chromosomes at this stage. It is evident that true double division, that is, double division of the chromosome thread, may happen, but the evidence that I have presented makes it probable that it is more uncommon than has been supposed. Where the chromosomes are too small to make direct observation possible, and where breeding behaviour does not supply auxiliary evidence, it is simpler to assume that the extra division is transverse, since this does not imply so great a departure from the normal meiotic process as an extra division of the chromosome thread.

4—THE ACTION OF THE CENTROMERE IN WALL FORMATION

We have seen that the centromeres react to the spindle in certain ways, and that the reaction of the centromere differs according to the time at which it comes under the influence of the spindle. It has further been suggested that the centromere has an effect upon the last stage of spindle action, namely, upon wall formation. Darlington (1936) suggests that after the chromosomes have separated at anaphase, the part of the spindle between them is no longer a centrosome spindle, controlled from the poles, but a centromere spindle, controlled by the separating centromeres. It follows from Darlington's assumptions, that if a univalent is left on the equator, midway between the separating groups, the reaction between its centromere and those composing the main spindle should determine a deflexion of the wall. The isolated chromosome should in other words become enclosed in a microcyte. An acentric fragment such as that accompanying a bridge should on the other hand not form a separate cell.

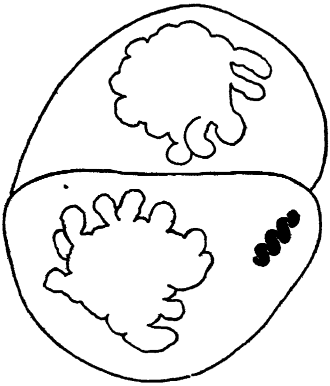

Fig. 25—Telophase of the first division in the diploid "Philippe de Commines". The fragment is acentric and consists of four left-handed coils. It is not cut off by a wall. It should be noted that the fragment, which has arisen as a result of crossing-over in an inversion, is composed of coils of a consistent direction. Changes of direction do not therefore necessarily occur in the cross-over chromatid at the point of crossing-over.

My observations on *Tulipa* confirm this. In diploids, where the only fragments present are acentric, microcytes have not been seen (fig. 25). In triploids, on the other hand, where whole chromosomes may be left on the equator, microcytes do occur (figs. 26, 27), although apparently not so commonly as in some organisms (e.g. *Hyacinthus*, Darlington 1929). When bridges are present the wall passes straight through them (figs. 22, 26). It is interesting to notice also that when a bridge is cut in this way, the two halves may be shifted, presumably by the growth of the wall, so that they do not exactly coincide (fig. 22). There must also be a shift of the entire bridge towards the edge of the spindle, for surviving first division bridges sometimes occur at the extreme edge of the cell in a position which they would never occupy at first anaphase (fig. 22). This must again be due to the growth of the new wall, accompanied by a change in shape of the spindle.

Frankel (1937) has examined diploid and triploid species of *Fritillaria*, also with this end in view. He finds that microcytes are common in the triploid species, but occur occasionally also in the diploid, which was an inversion heterozygote, forming acentric fragments quite commonly. It therefore appears that in certain circumstances the body repulsions of lagging fragments may bring about a deflection of the wall. Centric and acentric fragments act differentially only if cell walls are formed while the centromere is still active. Bridges cannot deflect the wall because they lie across the cell. There is in fact no way of avoiding them.

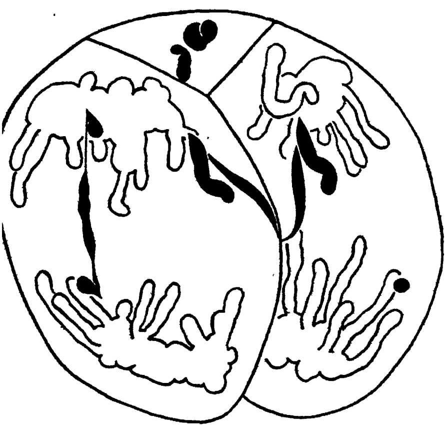

FIG. 26

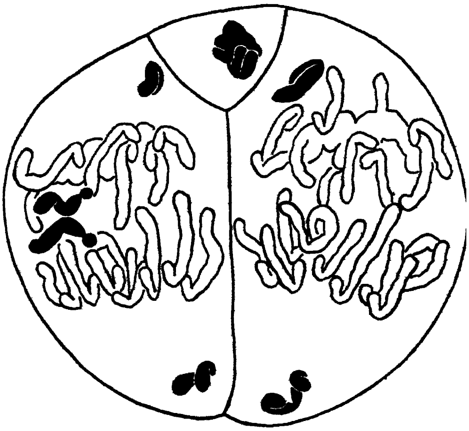

FIG. 27

FIG. 26—Anaphase of the second division in the triploid "Pink Beauty". A wall separates a microcyte containing two chromosomes, each of which appears to have a centric constriction. The cell also contains a first-division bridge passing through the wall, and a second-division bridge (cf. Pl. 14, *e* and *f*).

FIG. 27—The same, but here the chromosomes in the microcyte are degenerating. A pair of daughter univalents are lagging on either side of the wall. They must have divided at the previous anaphase (all × 2000).

The differences we find in the occurrence and frequency of microcytes as between diploid and triploid species of *Tulipa*, *Fritillaria* and *Hyacinthus* are evidently determined by differences in the activity of the centromere at the time when the walls are formed. Up to a certain stage in the reconstitution of the daughter nuclei, the centromere is the critical agent in modifying the shape of the spindle and controlling the formation of the new cell wall. At a later stage, this function may be taken over to some extent by the body-repulsions of the chromosomes or even by the surface of the nucleus (Darlington and Thomas 1937). The stage at which this occurs probably differs from one organism to another.

5—ABNORMAL MEIOSIS

Occasionally we find, in an otherwise normal anther, cells in which one or more of the characteristics of meiosis are absent. Two such cells, showing opposite modifications of meiosis, have been found. In the first, from the diploid garden tulip "Mrs. Moon" (fig. 28), the chromosomes exceed normal meiotic chromosomes in their degree of contraction, but no chiasmata have been formed. The distribution of the chromosomes in the cell suggests an irregular anaphase such as we might expect where failure of chiasmata had prevented congression. Yet the chromosomes resemble normal univalents at metaphase rather than at anaphase, for their constituent chromatids lie so close together that their double nature is concealed. Presumably this is because their chromatids are coiled in the same major spiral, as we find in bivalents of *Tradescantia* and other plants. Moreover, the centromeres have not divided as they sometimes do at the first anaphase in univalents, which again suggests that the cell is at metaphase.

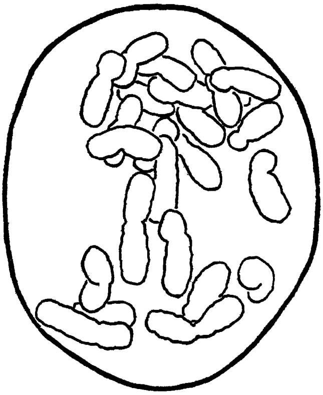

FIG. 28—Asynaptic cell in the garden variety "Mrs. Moon" ($2x$). No chiasmata have been formed, and all the chromosomes have the characteristic appearance of univalents at metaphase. They probably consist of two chromatids, but evidently the centromeres have not yet divided. (× 2000.)

The super-meiotic contraction shown by these chromosomes is often characteristic of cells in which there has been failure of chiasma formation. It occurs in asynaptic strains of *Avena* (Huskins and Hearne 1933), of *Zea*

(Beadle 1933) and of *Crepis* (Richardson 1935). Presumably in these cases there has been some dislocation of the normal time relations, so that pachytene pairing was prevented, perhaps by the early onset of splitting. This would give the chromosomes a longer time for contraction than in the normal.

The second abnormality is in many respects the reverse of this. This cell, from the triploid garden variety "Inglescombe Yellow" (fig. 29), is clearly at anaphase. The two daughter groups are separating normally with a number of univalents still lying on the equator (cf. fig. 8). But the chromosomes are abnormally long and thin, even longer and thinner than is usual at the second division of this variety (figs. 21, 22). This can hardly be due to their having uncoiled, for this process does not begin before telophase. We must then suppose that the coiling was arrested at an early stage of prophase or that there was a reduction in the usual rate. This anaphase must in fact have been derived from a metaphase similar to that found in *Fritillaria Meleagris* (Darlington 1936, IV, fig. 11) in which the usual number of chiasmata was present but in which the bivalents were of mitotic length.

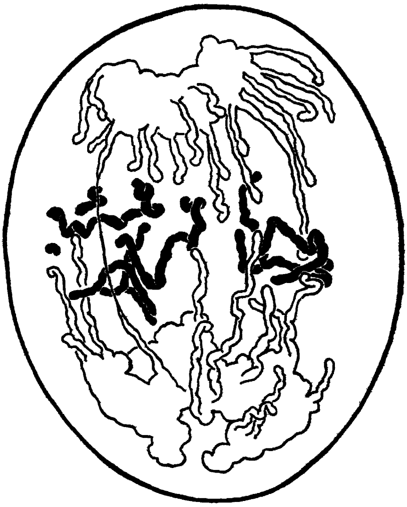

FIG. 29—Anaphase of the first division in "Inglescombe Yellow" ($3x$) in which the chromosomes are abnormally long and thin. Possibly this will result in the formation of a restitution nucleus. (× 2000.)

Whether the abnormal behaviour of these cells is the result of mutation or of reaction to external conditions peculiar to them cannot be determined, but the two possibilities must be borne in mind in considering such cases.

SUMMARY

1—At late anaphase of the first division of meiosis in some garden varieties of *Tulipa* which are heterozygous for inversions, constrictions appear next to the centromere, commonly in dicentric chromatids, and exceptionally in freely separating chromatids. They are presumably due to the stretching of the spindle. This stretching in its extreme form leads to the separation of the free sister arms of a dicentric chromatid at the centromere and indicates a special connexion between arms on opposite sides of the centromere.

2—In polyploids, univalents which come on to the plate at anaphase may be of three kinds: "true" univalents, or chromosomes which have not formed chiasmata; "open" univalents, or lagging members of multivalents; and "closed" univalents, or loop chromatids derived from a particular combination of chiasmata in an inversion and proximal to it. These three types may be recognized by their shapes and the positions of their arms.

3—Many of these univalents divide at the first division. In one variety the centromeres, as well as dividing in the normal direction, that is, longitudinally, may divide transversely, separating pairs of sister arms, or obliquely, separating three arms from one. Again the direction of division may be normal, but one or more arms may break away from the centromeres at an early or late stage of separation. If two arms break away, they may still remain connected to one another by a fine thread.

4—At the second division the daughter univalents may congress, in which case their centromeres may divide again. Since the chromosome thread has not divided, the only way in which this can occur is transversely, so that the chromosome fragments.

5—In *Tulipa* acentric fragments do not determine a deflexion of the first division wall, but whole chromosomes, if they lie midway between the poles, may do so, and give rise to supernumerary cells. In other words, the cell wall is formed under centric control.

6—These abnormalities show that the centromere has distinct properties of its own which govern not only the movements of the chromosomes but their structural continuity. These properties depend upon its special method and time of division, which unlike that of the chromomeres is not determined by its internal organization alone, but rather by its relationship with the spindle, with its chromatids and with other centromeres.

REFERENCES

Beadle, G. W. 1933 *Cytologia*, **4**, 269–87.
Belar, K. 1928 "Die cytologischen Grundlagen der Vererbung." Berlin.
— 1929 *Arch. EntwMech. Org.* **118**, 359–484.
Bennett, E. S. 1937 *Cytologia* (in the Press).
Darlington, C. D. 1929 *J. Genet.* **21**, 17–56.
— 1930 *J. Genet.* **22**, 65–93.
— 1932 "Recent Advances in Cytology." London.
— 1936 *Proc. Roy. Soc.* B, **121**, 264–319.
— 1937 "Recent Advances in Cytology", 2nd ed. London.
Darlington, C. D. and Thomas, P. T. 1937 *Ann. Bot., Lond.* (in the Press).
Erlanson, E. W. 1929 *Bot. Gaz.* **87**, 443–506.
Federley, H. 1931 *Z. Zellforsch.* **12**, 772–816.
Frankel, O. H. 1937 *J. Genet.* **34**, 447–62.
Huskins, C. L. and Hearne, E. M. 1933 *J. Roy. Micr. Soc.* **53**, 109–17.
Karpechenko, G. D. 1927 *Bull. Appl. Bot.* **17** (3), 305–408.
Katayama, Y. 1935 *Jap. J. Bot.* **7**, 349–80.
Kihara, H. 1931 *Cytologia*, **2**, 106–155.
Metzner, R. 1894 *Arch. Anat. Physiol.* (*Phys. Abt*), pp. 309–48.
Meurman, O. 1928 *Hereditas, Lund*, **11**, 289–356.
Newton, W. C. F. and Darlington, C. D. 1929 *J. Genet.* **21**, 1–16.
Nishiyama, I. 1931 *Jap. J. Genet.* **7**, 49–102.
Ribbands, C. R. 1937 *J. Genet.* **35**, 1–24.
Richardson, M. M. 1935 *J. Genet.* **31**, 119–43.
— 1936 *J. Genet.* **32**, 411–50.
Upcott, M. 1937 *J. Genet.* **34**, 339–98.

DESCRIPTION OF PLATE 14

All magnifications are × *ca.* 2000.

a–d. First meiotic anaphase in "Inglescombe Yellow". *a*, bridge in the short arm of one of the bivalents, showing two free long arms pulled apart at the centromere. *b*, univalent dividing so that the two daughter short arms each carry a centromere while the long arms do not (cf. fig. 19). *c*, normal univalent (left) and one with a closed loop chromatid (right). Neither has yet divided. *d*, loop chromatid dividing so that one centromere carries three arms and the other one (cf. fig. 17*b*).

e, *f*. Different levels of the same cell at the second anaphase in "Pink Beauty". It contains a first- and second-division bridge and a microcyte (cf. fig. 26).

612.621.5 : 577.176.52

Stimulation of the Corpora Lutea of the Rat by means of Progesterone and Testosterone

By T. McKeown and S. Zuckerman

Department of Human Anatomy, Oxford

(*Communicated by W. E. Le Gros Clark, F.R.S.—Received* 25 *August* 1937)

[Plates 15–17]

The administration of progestin or progesterone inhibits oestrus in normal female experimental animals and leads to the cessation of follicular growth (Papanicolaou 1926, working on guinea-pigs; Gley 1928, on rats; Corner 1935, on monkeys). Active testicular extracts or testosterone have a similar effect (Ihrke and D'Amour 1931; Lendle 1931; and Robson 1936, working on rats; Zuckerman 1937, on rhesus monkeys). There is evidence that a phase of luteal activity occurs whenever the ripening of follicles is inhibited (see Evans 1928; Weichert 1930, amongst others), and it was therefore of interest to enquire whether or not luteinization of the ovaries is also an effect of the administration of progesterone and testosterone to normally cyclic female rats. Previous reports on this question are negative. Papanicolaou (1926) observed degenerative changes in the ovaries of guinea-pigs in which several successive oestrous cycles had been suppressed by means of progestin. Selye, Browne and Collip (1936) injected six rats with 4 mg. of progesterone for 12 days, and at the close of the experiment found that neither recent corpora lutea nor mature follicles were present in the ovaries.

MATERIAL AND METHODS

Albino and black and white rats were used. All injections were given once daily and subcutaneously. Oestrone was administered in aqueous solution, and both progesterone and testosterone propionate in oil. At the end of each experiment the uterus and one of the ovaries were fixed in Bouin's fluid, and after sectioning, stained with haematoxylin and eosin, the ovary being sectioned serially. The second ovary of each animal was fixed in Flemming's chrome-osmium fluid with acetic acid, and prepared to show osmicated fats according to the technique described by Deanesly (1930). In certain cases, noted below, the uterus was traumatized by the method of Shelesnyak (1933*a*) (a modification of that of Long and Evans), in order to produce deciduomata.

EXPERIMENTAL

1—*The effect of testosterone on the ovary*

Six normally cyclic rats (R. 29–31, 34–36) were injected daily for 10 days with 0·2 mg. of testosterone propionate. Two of the rats were in dioestrus, two in prooestrus and two in oestrus at the start of the experiment, during which they all passed into, and remained in dioestrus. On the sixth day a needle was passed through both uterine horns of each animal. Autopsies were performed on the tenth day.

The ovaries of all the animals contain several normal and presumably functional corpora lutea (cf. fig. 1, Plate 15). The corpora are of various sizes, each ovary having one or two with diameters of 1·5 mm., the largest seen being 1·8 mm. in diameter. Long and Evans (1922) give the following maximum dimensions for the normal corpora lutea of the rat:

Corpora lutea of ovulation	... ...	1·2 mm.
„ „ pseudopregnancy	...	1·4 mm.
„ „ pregnancy	... ...	2·0 mm.
„ „ lactation	... ...	1·4 mm.

The constituent cells of the healthy corpora are large, and have relatively small nuclei and distinct cell boundaries (fig. 2, Plate 15). The cytoplasm is not vacuolated, and is usually free of large fat granules. Older corpora are to be seen in all the ovaries. Their constituent cells are shrunken and show other signs of degeneration. Many of the fresh corpora are not quite solid (cf. figs. 1 and 3, Plate 15), and strands of lutein cells are to be seen encroaching on the remains of the antrum. None of the ovaries contains large follicles.

Each corpus luteum was examined in serial section. In many cases it seems plain that the lutein cells had developed from the granulosa of ruptured follicles (cf. fig. 3, Plate 15, which shows an incompletely organized corpus luteum with a rupture point bridged over by restored germinal epithelium). Long and Evans (1922) state, however, that solid corpora lutea in the normal rat ovary are frequently corpora lutea atretica, even though no sign can be found of the retained ova. The possibility that some of the corpora in our specimens are corpora lutea atretica cannot therefore be excluded, and the fact that many well-organized and apparently healthy corpora are very small may be taken to indicate that they were formed by the luteinization of small unruptured follicles.

Further evidence that functional corpora lutea were present in the rats injected with testosterone propionate is provided by the condition of their uteri. All the uterine horns were swollen, and in one instance (R. 34) the enlargement was considerable. Microscopic examination of the latter specimen shows a well-developed placentoma (figs. 4 and 5, Plate 16), with typical decidual cells in process of rapid division. The remaining specimens have endometria of the type shown in fig. 6, Plate 17. The surface and glandular cells are tall columnar, and in most cases are actively secreting. The epithelium lining the cavum uteri is much folded, and some of the endometrial glands are greatly distended. In two specimens there are masses of leucocytes in both the glands and the cavum uteri. The character of the stroma varies from animal to animal. In some it is more cellular and vascular than in others, and the stromal nuclei are often very swollen. This picture corresponds almost exactly to that described by Deanesly and Parkes (1931) for the sterile horn of the mouse in the second half of pregnancy. To our knowledge it has not been found in a normal reproductive phase of the rat. That the changes were not a direct effect of testosterone on the endometrium is shown by two groups of experiments described below.

Selye and McKeown (1935) state that the decidual reaction can only be obtained when the endometrium is in a less advanced phase of growth and secretion than that just described. It is therefore likely that deciduomata were not obtained in five of the six rats discussed above because they had already passed beyond the "first progestational reaction" (Selye, Collip and Thomson 1935) when trauma was applied to the uterus. Another possible explanation is that the luteal stimulation of the endometrium was not entirely normal. It is well known that the conditions under which placentomata can be formed are very specific and sensitive (e.g. see Long and Evans 1922; Shelesnyak 1933*b*). Parkes (1929), for example, failed to stimulate placentomata in X-irradiated rats whose ovaries were luteinized by means of A.-P. extracts.

2—*Uterine sensitivity in spayed rats treated with testosterone propionate*

In view of the finding that testosterone may exercise slight progestational effects on the endometrium (Klein and Parkes 1937), it became necessary to enquire whether the endometrial changes demonstrated in the previous set of experiments were in fact produced by way of the ovaries, or whether they resulted directly from the action of testosterone. Accordingly five normally cyclic rats were spayed (R. 37–41), and a 9-day course of testosterone propionate injections (0·2 mg. daily) begun on the same day. On the fifth or sixth days of the experiment, the uteri were traumatized. Autopsies were performed on the ninth or tenth days. At the start of the experiment two of the animals were in oestrus, two in prooestrus, and one in dioestrus.

No decidual or obvious progestational changes have taken place in any of the uteri (fig. 7, Plate 16). They are all smaller in size than those of the previous set of experiments. The glandular epithelium is neither high nor secreting. The stromal nuclei are occasionally swollen.

Since decidual reactions are obtained in spayed rats injected with progestin only if they have first been injected with oestrin (Weichert 1928; Nelson and Pfiffner 1930; Shelesnyak 1933 *b*), the experiment was repeated on six more ovariectomized rats (R. 42–47) which were first injected daily for 4 days with 14 I.U. of oestrone. The results were again negative, no decidual or obvious progestational changes being produced (fig. 8, Plate 17). One of the specimens is peculiar in being exceptionally glandular. Its epithelium, however, is inactive.

These negative results suggest that the decidual and progestational changes obtained in the first set of experiments were dependent on the ovaries, and presumably on the corpora lutea they contained.

3—*The effect of progesterone on the ovary*

Seven normally cyclic rats (R. 24–28, 32, 33) were injected daily for from 9 to 11 days with 1 mg. of progesterone. Autopsies were performed on the last day of injections. Three animals were in prooestrus, two in oestrus, and two in dioestrus at the start of the experiment. With the exception of one animal (R. 32) all the rats passed into and remained in dioestrus during the course of the experiment.

Apart from those of R. 32, the ovaries of all the animals treated in this way contain several healthy corpora lutea similar to, but as a rule slightly smaller than, those seen in the first group of experiments (figs. 1, 2 and 3, Plate 15). R. 32 came into oestrus on the sixth day of injections. Its ovaries contain several follicles and less recent luteal tissue than the other specimens.

No attempt was made to secure a decidual reaction in this group of animals, since any positive results that may have been obtained could have been interpreted as a direct effect of the injected progesterone, and not as an effect of the hormone produced by the corpora lutea. The uteri of these animals are about the same size as those of the second and third sets of experiments, and much smaller than those of the first. Moreover, the growth and secretory changes which they exhibit (fig. 9, Plate 17), while fairly distinct in some, are in general much less advanced than those manifested by the group of normal rats which in addition to injections of testosterone propionate were subjected to uterine trauma. This difference may either be attributed to the trauma, or to the fact that the corpora lutea were larger in the group of animals that had been injected with testosterone.

Discussion

The above experiments show that the dioestrus which follows the administration of testosterone or progesterone to normally cyclic female rats is associated with the formation of functional corpora lutea. Whether the ovarian change results from the suppression of follicular growth, or whether it is a direct effect of the injections, cannot be decided on the available evidence. Zwarenstein (1936), Shapiro (1936) and Shapiro and Zwarenstein (1937) have found that progesterone and testosterone will cause ovulation in the normal or hypophysectomized South African "clawed toad" (*Xenopus laevis*), as well as in the excised ovary of this animal. Differences undoubtedly occur in the responses of the gonads of different classes of vertebrate to stimulation by gonadal hormones, but the fact that the amphibian ovary reacts in this way clearly suggests the possibility that the luteinization observed in our own experiments was a direct effect of the injections, and not an indirect one due to the suppression of follicles.

As noted above, both obvious small corpora lutea atretica and fresh corpora lutea that had developed from ruptured follicles are present in our specimens. Some of the larger corpora presumably date from ovulations which occurred at the start of the experiments, when the animals were not completely under the influence of the injected hormones. Many corpora lutea are, however, not quite solid (cf. figs. 1 and 3, Plate 15), and it is necessary to bear in mind the possibility that new corpora lutea were formed late in the experiments.

We wish to express our best thanks to Dr. K. Miescher, of the Ciba Company, for the testosterone propionate, and to Dr. M. Tausk, of the Organon Company, for the progesterone used in this study.

SUMMARY

Normally cyclic female rats were injected with testosterone propionate or progesterone daily for from 9 to 11 days. At autopsy several recent corpora lutea were seen in the ovaries of all the experimental animals. The activity of these corpora is indicated by progestational changes in the uterus and by the production of a deciduoma in one of the rats that had been injected with testosterone. Similar endometrial changes were not induced in spayed rats that were injected with oestrone followed by testosterone, or with testosterone alone.

REFERENCES

Corner, G. W. 1935 *Amer. J. Physiol.* **113**, 238.
Deanesly, R. 1930 *Proc. Roy. Soc.* B, **106**, 578.
Deanesly, R. and Parkes, A. S. 1931 *Proc. Roy. Soc.* B, **109**, 196.
Evans, H. M. 1928 *Amer. J. Physiol.* **85**, 149.
Gley, P. 1928 *C.R. Soc. Biol., Paris*, **98**, 504.
Ihrke, I. A. and D'Amour, F. 1931 *Amer. J. Physiol.* **96**, 289.
Klein, M. and Parkes, A. S. 1937 *Proc. Roy. Soc.* B, **121**, 574.
Lendle, L. 1931 *Arch. exp. Path. Pharmak.* **159**, 463.
Long, J. A. and Evans, H. M. 1922 *Memoirs Univ. Calif.* **6**, 1.
Nelson, W. O. and Pfiffner, J. J. 1930 *Proc. Soc. Exp. Biol., N.Y.*, **27**, 863.
Papanicolaou, G. N. 1926 *J. Amer. Med. Ass.* **86**, 1422.
Parkes, A. S. 1929 *Proc. Roy. Soc.* B, **104**, 183.
Robson, J. M. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **35**, 49.
Selye, H., Browne, J. S. L. and Collip, J. B. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **34**, 472.
Selye, H., Collip, J. B. and Thomson, D. L. 1935 *Endocrinology*, **19**, 151.
Selye, H. and McKeown, T. 1935 *Proc. Roy. Soc.* B, **119**, 1.
Shapiro, H. A. 1936 *Chem. Industr., Berl.*, **55**, 1031.
Shapiro, H. A. and Zwarenstein, H. 1937 *J. Physiol.* **89**, 38 *P.*
Shelesnyak, M. C. 1933*a* *Amer. J. Physiol.* **104**, 693.
— 1933*b* *Anat. Rec.* **56**, 211.
Weichert, C. K. 1928 *Proc. Soc. Exp. Biol., N.Y.*, **25**, 490.
— 1930 *Physiol. Zoöl.* **3**, 461.
Zuckerman, S. 1937 *Proc. Roy. Soc.* B, **123**, 457. *Lancet*, ii, 676.
Zwarenstein, H. 1936 *Proc. Roy. Soc. S. Afr.* 21 Oct.

EXPLANATION OF PLATES 15–17

FIG. 1—Ovary of R. 33, an animal that had been injected with progesterone, showing corpora lutea. × 17.

FIG. 2—Lutein cells in ovary of R. 27, an animal that had been similarly treated. × 240.

FIG. 3—A corpus luteum in ovary of R. 26, which had also been injected with progesterone, showing antrum filled with follicular fluid and blood, and rupture point bridged over by reformed germinal epithelium. × 78.

Fig. 4—Deciduoma in R. 34, a rat that had been injected with testosterone propionate. × 21.

Fig. 5—High power view of decidual cells in R. 34. × 226.

Fig. 6—Endometrium of R. 36, which was also injected with testosterone propionate, showing changes similar to those that occur in the sterile horn of mice in advanced pregnancy. × 21.

Fig. 7—Uterus of R. 39, an animal that was given a course of testosterone propionate injections beginning on the day it was ovariectomized. × 21.

Fig. 8—Uterus of R. 45, a spayed rat that was treated in the same way as R. 39 after 4 days' injection with oestrone. × 21.

Fig. 9—Uterus of R. 32, a normal rat that was injected with progesterone. × 21.

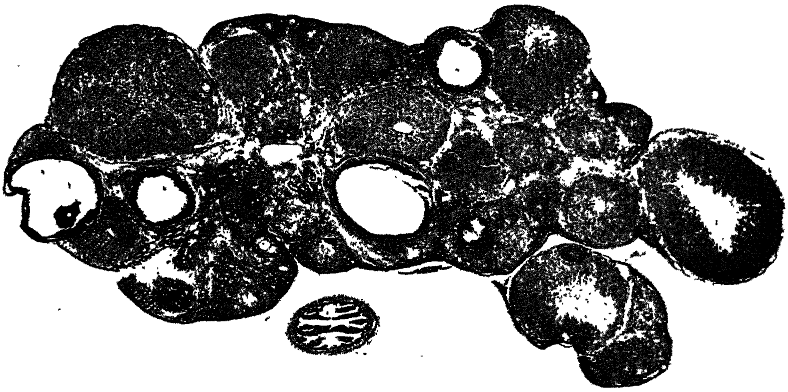

Fig. 1

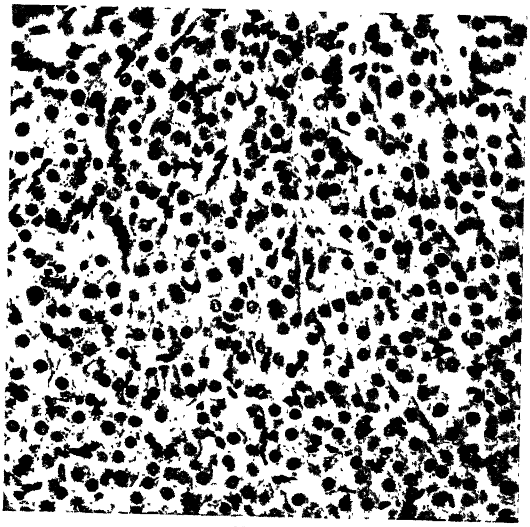

Fig. 2

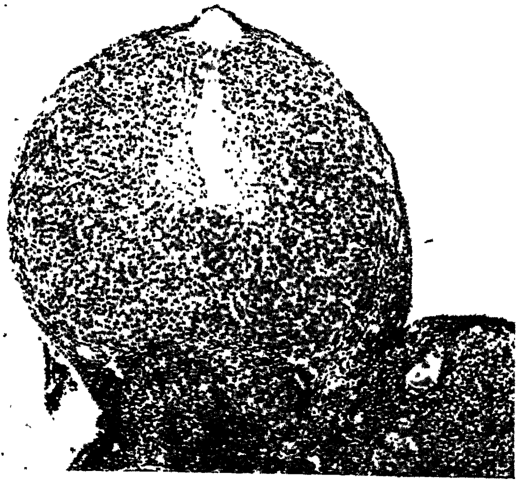

Fig. 3

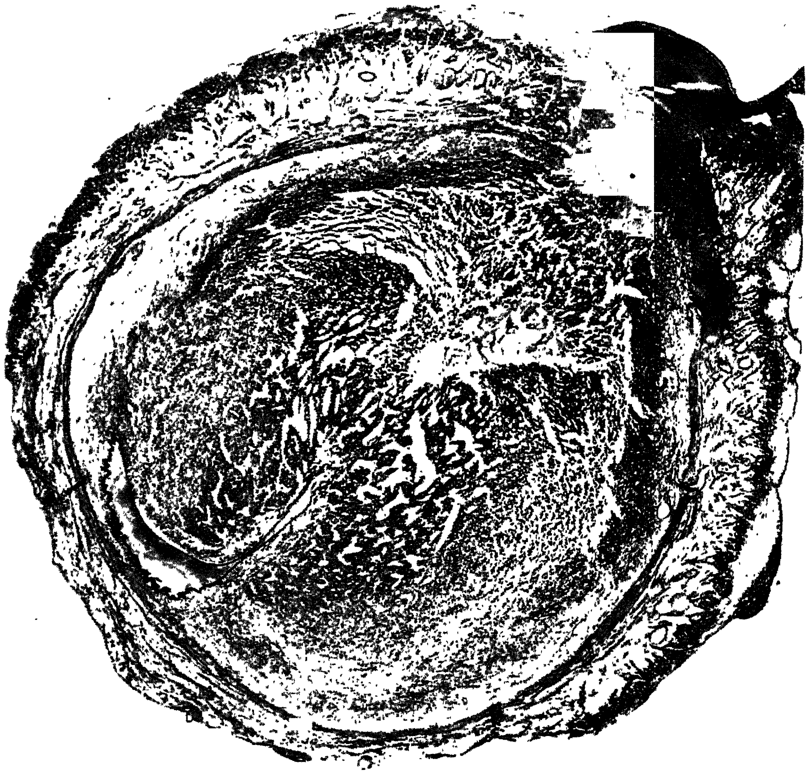

Fig. 4

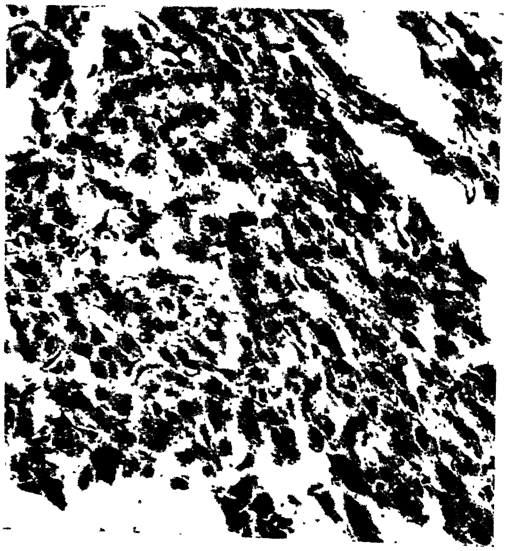

Fig. 5

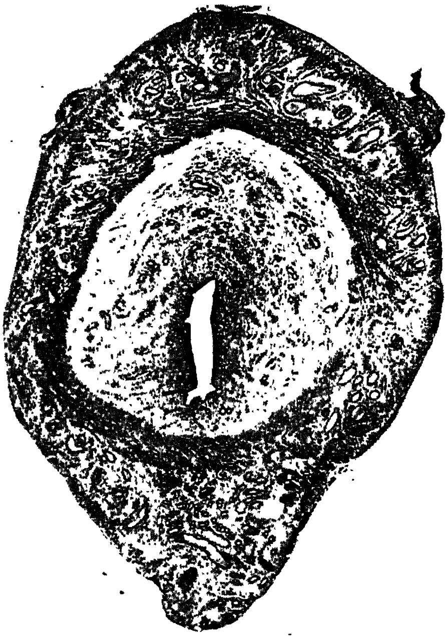

Fig. 7

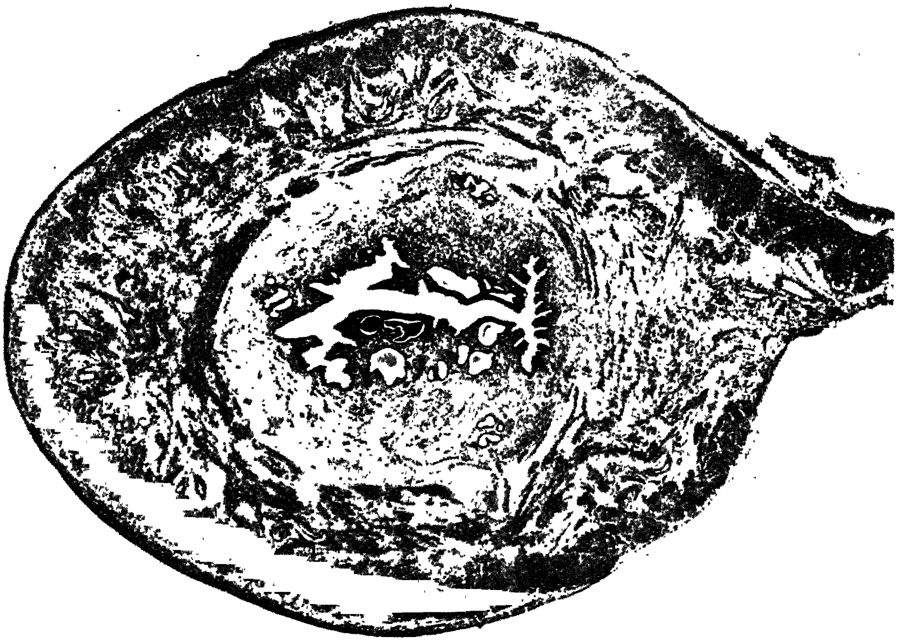

FIG. 6

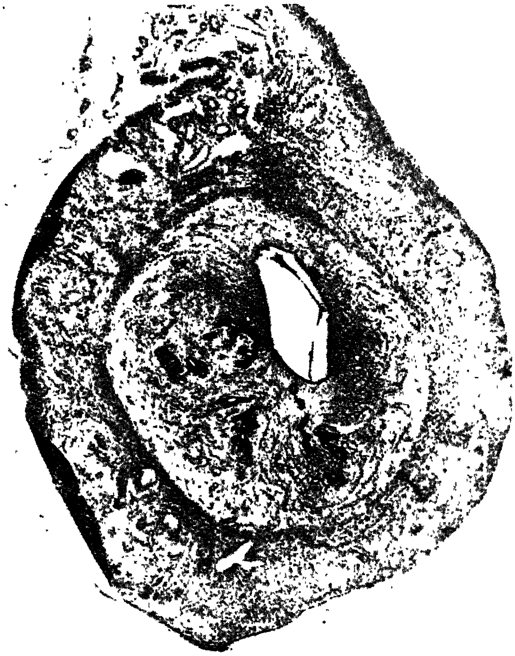

FIG. 8

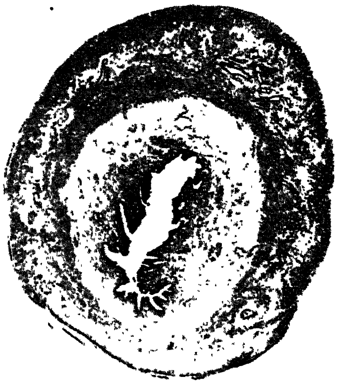

FIG. 9

Address of the President Sir William Bragg, O.M., at the Anniversary Meeting, 30 November 1937

This year the list of those whom death has taken from our Society is very long and heavy.

John Theodore Cash (1854–1937) was a Fellow of the Society for nearly fifty years. He was very active in research during the eighties and nineties of the last century, and his work helped materially to characterize British pharmacological research of that period. The results of his more important researches were contributed to the Society. They were mainly concerned with the connexion between chemical constitution and pharmacological action and were made in conjunction with the late Sir Lauder Brunton and afterwards with Professor Dunstan. His name will always be associated with the pharmacological action of organic nitrites and of the various aconitines prepared by Dunstan. But his contributions ranged over a wide field, and many were made to other Societies and Associations. All his work was done with enthusiasm and with most painstaking care. These characteristics were shown in his various activities in connexion with the great war. Personally he was of a somewhat retiring disposition but was a charming companion for those who were favoured with his friendship. His chief recreation was salmon and trout fishing, and to a former generation he was widely known as an expert angler.

Edgar Hartley Kettle, Professor of Pathology at the London Postgraduate School of Medicine, died December 1, 1936 at the age of 54. He was successively, pathologist to the Cancer Hospital, Fulham, Lecturer on Pathology, St. Mary's Hospital, Professor of Pathology at Cardiff, at St. Bartholomew's Hospital, and from 1934 till his death at the London Postgraduate School at Hammersmith. The subjects of his contributions to pathology range from tumours to silicosis. His work on the latter subject and on the effects of noxious dusts, important in industry, are a permanent contribution to knowledge, and the experimental methods he devised for their investigation have improved our resources in the study of this difficult and economically important field. Kettle insisted on the specificity of the lesions produced in the tissues by irritants, whether microbic or inanimate, and showed in his work how these changes could be used as indicators for their presence and causal importance in disease.

In the various schools in which he worked Kettle showed his personal gifts of teaching and administrative ability, and his tact and balanced judgement made him friends everywhere. His election to the Fellowship in May 1936, six months before his death, was welcomed by pathologists throughout the country. His premature death is a loss to the Society as well as to scientific medicine.

Sir HERBERT JACKSON (1863–1937) has been perhaps most widely known for his work, during the great war, on "Chemical and Optical Glasses", the national importance of which has tended to distract attention from his investigations into phosphorescence and phosphorescent materials, the chemistry of photographic processes, his early work in the X-ray field, and so forth.

Primarily, Jackson was an experimenter. Under Bloxam he received a thorough training as an organic chemist, and during his subsequent career he applied his chemical knowledge and his extraordinary energy and experimental skill to the investigation of any problems which came or were brought to his attention.

He was also a microscopist of the first rank, and his microscope was in constant use to assist him in the various investigations which he undertook during a singularly active and varied career.

He published little and kept only the scantiest of notes, but the variety and extent of the work which he did could be gauged by the wealth of knowledge and experience which he could bring to bear on matters of the most diverse character, and by the esteem in which his opinion and advice were held by all who had occasion to call upon him for assistance.

The career of ALFRED ARTHUR ROBB (1873–1937) was entirely devoted to intensive study and investigation. Even at school and College the original trend of his thoughts interfered with his record of success along the normal lines. After graduating at Belfast and later at Cambridge he passed on to Göttingen, where under the influence of W. Voigt he became immersed in the theory of the spectromagnetic effect of Zeeman, then passing from its simple origins into complex developments which are now fundamental, and his degree thesis was for that time a notable analysis of the subject. But his later interests were on the philosophical side. Ever since the days of the great astronomer Bradley, a friend of Newton, there had been a latent discrepancy in the relations of time and space arising from the time required for light to travel, which has only recently been tackled at length, mainly along the impressive lines developed by Alfred Einstein. This, which had become experimentally prominent under Michelson and Rayleigh, was just the kind of subject to excite Robb. His way of probing it to the bottom was from a postulate that the ultimate formative element in the cognition of every observing mind was simply linear succession of impressions in time, named by him the principle of before and after, and he had already advanced and perhaps established the thesis that in order that these impressions of various observers throughout the universe should be mutually consistent, the events to which they relate must be arranged in spatial order not, however, confined to the simple Euclidean space. The mode of argument comes out perhaps most simply in the introductory chapter of the recent second edition of his main book, which he named a "Theory of Space and Time".

Sir GRAFTON ELLIOT SMITH was born in Australia in 1871. After a distinguished early career at the University of Sydney, he entered St. John's College, Cambridge, in 1896. There he obtained a Fellowship in 1899 and was elected an Honorary Fellow in 1931.

In 1900 he was elected to a Chair of Anatomy in the Egyptian School of Medicine in Cairo. From Cairo he was called in 1909 to occupy the Chair of Anatomy in the University of Manchester. After ten fruitful years of work there, he succeeded Sir George Thane in the Professorship of Anatomy in University College, London, where he remained until his retirement in September 1936.

In 1907 he was elected into the Fellowship of this Society, in which he served twice on the Council and also as Vice-President in 1913. He was awarded a Royal Medal in 1912. He received the honour of Knighthood in 1934, and in 1936 the Decoration of Chevalier de l'Ordre National de la Légion d'Honneur.

Elliot Smith's contributions to the subject of brain morphology, both in the way of ascertained fact and in interpretation, were such as to give his work a permanent place in the development of neurological science. He holds a high place for contributions to anthropological science, such as his studies on mummification and on the problem of Egyptian origins: perhaps even more important were his studies in the problem of human ancestry in general. To many of his contemporaries, especially in his later period, he was probably best known as an apostle of what is known as the "diffusionist school" in reference to human cultural origins. Around this phase of his activity controversy has been keen and vocal, his own voice being by no means lacking in resonance.

No account of Elliot Smith, however brief, must fail to take note of his essentially lovable disposition, never quenched by the clamour of the arena.

Nor would any account of him be at all adequate which lost sight of the enthusiasm which he inspired in his pupils and co-workers, for whom he was, and remains, a memorable personality.

On January 13 of this year ROBIN JOHN TILLYARD met an accidental death in Australia. Born in 1881, his early training and career were mathematical. His dominant interest in later years was in animal life, and he became a professional entomologist. After eight years on the staff of the Cawthron Institute, Nelson, New Zealand, he became, in 1928, Commonwealth Entomologist for Australia. He held this post only six years when ill-health forced his retirement. His early biological work was on dragonflies, upon which he wrote many important papers. These researches led him to study the broader problems of wing venation, to the solution of which he made significant contributions. These in turn prepared him for the investigation of the highly interesting fossil insects which were then being disclosed, and his many contributions in this field made him the leading exponent of his day. He also wrote extensively on the more primitive orders of insects living in the Antipodes, and he was the author of a large textbook on the insects of Australia and New Zealand. In economic entomology he achieved notable success in applying biological control to the woolly aphis of the apple in New Zealand. Tillyard became a Fellow of the Royal Society in 1925.

JOHN ALEXANDER MACWILLIAM (1857–1937) studied at the Universities of Aberdeen, Edinburgh and Leipzig, and at University College, London. From 1886 to 1927 he held the Regius Chair of Physiology at Aberdeen. He spent his life investigating the circulatory system, particularly the regulation of the activity of the heart. His early inspiration was drawn from Dr. W. H. Gaskell of Cambridge, and he owed much to his training at the famous school of Ludwig at Leipzig. His researches were at times interrupted by indifferent health, but in spite of that he published important papers which have won him a great reputation. He was a gifted lecturer. He was elected to our Society in 1916.

MAX WEBER, who died last February in his eighty-fifth year, was elected a Foreign Member of our Society in 1935. He was a famous teacher, who taught the whole present generation of Dutch zoologists; and he was himself the last great example of the old school of naturalists who took the whole world for their field of study. As a comparative anatomist he did for our day what Owen did for mammalian anatomy in his; as a student of fishes he set in order the whole rich fauna of the East India Seas; as a traveller and explorer he brought home from the north and the south and the east immense collections for his brother naturalists and himself to study. He was a man of wide culture and great distinction, honoured and beloved throughout the whole brotherhood of zoologists.

Francis Sowerby Macaulay, born near Oxford and educated at Bath, graduated from St. John's College, Cambridge, in 1882, and after some time at his old school settled down for many years as chief mathematical master at St. Paul's School. That he was a schoolmaster of novel and quite outstanding ability is shown by the long list of distinguished men whom he sent out. On retirement he settled in Cambridge, was made F.R.S. in 1928, and though hampered by increasing weakness in his last years, continued his interest in his work and in the work of younger men to the end.

He was a man of penetrating logical mind, and was attracted while still a schoolmaster by the algebraical difficulties surrounding a formula, given by Max Noether, for the expression of the equation of a plane curve passing through the intersections of two given curves. This study, to which he devoted many published papers, led him to the study of modular systems, that is, systems of algebraical polynomials which are expressible linearly by means of definite polynomials (with coefficients suitably chosen polynomials). The work had a very living interest in connexion with Hilbert's remarkable proof for the finiteness of the system of invariants of a binary form; this question of finiteness had been a major preoccupation for Cayley and Sylvester and Paul Gordan over many years. Macaulay developed his investigations into a Cambridge Mathematical Tract (1916), which remains a striking contribution to a subject little known in this country. His later writings were for the most part developments of this earlier work.

When William Taylor, who was elected to our Society in 1934, died in February of this year, the country lost a very great designer and organizer in the realm of fine engineering and optical instruments. As the moving spirit in his firm during the half-century from 1887 to 1937 he developed the design of their precision products, and organized the manufacture of camera lenses of high quality, until Taylor, Taylor and Hobson and their products were known in every country of the world.

But he had more important, though less spectacular, works to his credit. By his work on Government, British Association and other Committees, he materially raised the standard of mechanical and optical manufacture in this country.

As a designer he showed a comprehensive knowledge of engineering practice, an authoritative grasp of the result to be desired, and an untiring patience till his goal was reached.

These qualities were equally brought into play in the running of his factory, which indeed seemed in its arrangements to be characterized by the same accuracy and beauty of design as were displayed in the objects which it produced.

Sir HENRY CAPEL LOFFT HOLDEN was born in 1856, was educated at Ipswich School and the Royal Military Academy. He entered the Royal Artillery in 1875, served in India and was subsequently Superintendent of the Royal Gun and Carriage Factories at Woolwich. As a serving officer he was instrumental in introducing scientific methods into Woolwich Arsenal, particularly in all that concerned the testing of guns and explosives. He possessed a thorough practical knowledge of electricity, and considerable inventive genius. He was a keen experimenter, who was much aided in the design of the various instruments which he invented by his very highly developed skill as an amateur mechanic. The proof butts at Woolwich were first equipped with an efficient modern instrument system as a result of his activities, and the Holden chronograph is still in use for the determination of muzzle velocities. His technical services were also utilized by the War Office in the reorganization and re-equipment of the Royal Gun Factory and the Royal Carriage Factory. The technical excellence of British gunnery during the great war was largely due to his belief in the need for the systematic application of science to technical problems.

SYDNEY YOUNG died on April 9 in his eightieth year. Having worked under Roscoe and Schorlemmer at Owens College he was appointed as Ramsay's assistant in 1882. A long series of papers has linked the names of Ramsay and Young, and set a standard of excellence in physico-chemical determinations. They undertook a systematic investigation of the vapour pressures of solids and liquids, the thermal properties of pure liquids, and the thermodynamical relations involved. Succeeding Ramsay in the Chair at Bristol, Young continued with undiminished energy and, with his pupils, studied the critical constants of pure liquids. The Society's Fellowship, bestowed at the age of 36, was a recognition of the value of this work. The necessity of having liquids of the highest purity led him to study methods of purification by fractional distillation, and it is for this work, purification by means of the formation of binary and ternary mixtures of constant boiling-point, that his name is best known. Along these lines his work found numerous industrial applications. From 1904 to 1928 he held the University Professorship of Chemistry at Trinity College, Dublin. There teaching duties left little time for research, but he published several articles and two important books.

Arthur Harry Church, whose whole life as a working botanist was spent in the Department of Botany at Oxford, combined remarkable distinction of mind with a peculiar mentality, not free from a certain aloofness, which tended to cut him off from his fellow-botanists. He never followed beaten tracks, nor devoted himself to "fashionable" work, but always took his own line, reconsidering many problems in an original way on the basis of patient, long-continued and penetrating investigations. His primary interests were in morphology, and his powers of observation and delineation of plant structures were of the highest order. His theoretical writing was sometimes one-sided, but always valuable and stimulating, and while some of his views have not been and are not likely to be accepted, he illuminated many topics by his fresh treatment and comprehensive outlook. Probably the work least open to criticism is his study of phyllotaxis, which undoubtedly placed the subject on a sound basis. Much of his long paper on "Thalassiophyta", in which he dealt with the condition of evolution of plant life in the sea and its later adaptation to land conditions, is extremely interesting and valuable. Some of Church's best work—notably on the morphology of the flower—was never published, but remains buried in notes for his students. As a teacher he was pre-eminent, and many generations of Oxford undergraduates remember with deep gratitude the stimulus of his teaching, at once critical and constructive. Probably Church had a wider knowledge of the facts and problems of botany—other than the purely physiological—than any teacher of his generation; and he left a deep and lasting impression on the Department in which he laboured continuously and devotedly for more than 30 years.

Arthur George Perkin was born in 1861, a member of a family devoted to chemical research. He worked under Franklin and Guthrie at the Royal College of Science and subsequently in the Dyeing Department of the Yorkshire College at Leeds. For ten years he was with a Manchester firm, manufacturing alizarin, and had been for some time manager when he left in 1892 to join the Yorkshire College as lecturer and research chemist. In 1916 he succeeded A. G. Green as Professor of Colour Chemistry.

Perkin's fame rests securely on his fine contributions to our knowledge of natural and synthetic colouring matters. He wrote a very large number of papers of importance; he contributed many of the articles on natural dyes in Thorpe's "Dictionary", and with A. E. Everest he published in 1918 a standard work on "The Natural Organic Colouring Matters".

Perkin was an able and inspiring teacher. Though his ways were gentle, his influence was profound. He was very successful in fitting men for industrial work, and the department over which he presided in the University of Leeds acquired a high reputation as a centre both of knowledge and training. His work has been of national importance. He became a member of our Society in 1903, and in 1924 he was given the Davy Medal, which he thoroughly deserved.

The career of THOMAS MATHER illustrates the capacity-catching value of Whitworth Scholarships. One of the first of these was gained by Mather in 1878 after a strenuous five years' engineering apprenticeship combined with regular attendance at evening classes. He was thus enabled at the age of 22 to enter a four-years College course, through which he passed with great distinction. He became an assistant to Professor Ayrton, first at Finsbury and afterwards at South Kensington. On the death of his chief he succeeded him as Professor of Electrical Engineering.

He began to teach when electrical engineering was an entirely new industry. His main life work consisted in the initiation and development of teaching appliances suitable for technical students and also in the help which he gave to laboratory workers of all grades. He was an able pioneer both in the College work which had to precede the commercial production of good instruments and in the equipment of laboratories for industrial testing and research.

The value of his work was recognized by his election as F.R.S. in 1902. Six years later he was joint author with Professor Ayrton and F. E. Smith of three important papers published by the Royal Society and dealing with the establishment of the National Electrical Standards.

The death of HENRY EDWARD ARMSTRONG on July 13, in his ninetieth year, deprives our Society of its senior Fellow (his election dating so far back as 1876), and many scientific institutions and assemblies of a familiar and distinguished figure. When the Davy Medal of the Society was presented to Professor Armstrong in 1911, in recognition of his researches in organic and physiological chemistry, reference was made to the active part he took in the discussion and criticism of current theories in chemistry, and to the fruitful fields of inquiry which he originated or in which he co-operated.

Throughout his scientific life, the spirit of the motto of our Society was manifest in him; and his judgement was never influenced by authority. An example of his departure from orthodox methods was the introduction of crystallography into the course of practical chemistry at the City and

Guilds College, fifty years ago, in association with Sir Henry Miers. This largely determined the character of important later researches by his two assistants, Sir William Pope and Professor T. M. Lowry, both of whom became Fellows of our Society. Four other Fellows who were his colleagues or students, and whose work he inspired, are Professor W. P. Wynne, Professor Stanley Kipping, Professor A. Lapworth, and his son, Dr. E. F. Armstrong. His students occupy leading positions in science and industry in many parts of the world; and in August 1927 they presented to him and Mrs. Armstrong his portrait by Mr. T. C. Dugdale, exhibited at the Royal Academy in that year.

Professor Armstrong was an acute observer and inquirer in many scientific fields other than that of chemistry, such, for example, as agriculture, botany and geology, and he was an untiring advocate of the introduction of research methods into science teaching at all stages.

GEORGE ADOLPHUS SCHOTT (1868–1937) was educated at Bradford Grammar School and Trinity College, Cambridge. He was for a long time Head of the Department of Applied Mathematics at Aberystwyth. The solution of the Hertz-Heaviside form of Maxwell's equations corresponding to the general motion of a point charge is effected by means of the retarded potentials of Liénard. Except in the simplest cases, such as uniform rectilinear motion and in the case of quasi-stationary motion, the evolution of these potentials presents a problem of extreme complexity. Schott was excelled by none in finding out cases capable of explicit expression in elliptic functions and in obtaining Bessel expansions of great generality. His Adams Prize essay, which was published in 1912 under the title "Electromagnetic Radiation", seemed to contain the utmost contribution of the classical theory to the radiation problem. The advent of the Bohr theory, however, turned his attention to the possibility of radiationless orbits, and it is gratifying to know that he crowned his long researches on this subject by actually discovering such orbits within the scope of the classical theory. His last paper containing this discovery was published in the *Proceedings of the Royal Society* in April of this year. In size such orbits were much smaller than the Bohr orbits, and sadly enough in his last paper he speaks of future developments to follow. What possibilities lie in Schott's orbits is a matter for the future.

Among those who drew their inspiration from, and furthered, the pioneer work of Cayley, Sylvester and Salmon in the algebraic invariant theory in the nineteenth century, EDWIN BAILEY ELLIOTT takes a very distinguished place. His peculiar gift of analytical manipulation of complicated mathematical structures was governed by a rare insight and feeling for the natural approach and the appropriate line of enquiry. Continental mathematicians, notably Gordan and Hilbert, had advanced the invariant theory by discoveries of capital importance as to the finiteness of certain apparently infinite systems, this stage being completed in the year 1890. Elliott, who already had shown plain signs of his analytical skill, thereafter became the chief interpreter of these newer concepts in terms of the original operation and theories of Sylvester. His book—the "Algebra of Quantics"—which first appeared in 1895, is a model of its kind, and could not be bettered for lucidity and charm. It is an admirable introduction to his own considerable original writings, and those of his forerunners. His latest published work lay in the field of pure analysis, where he produced a beautiful and simple proof of a theorem on the convergence of positive terms that had taxed the ingenuity of leading analysts. Professor Elliott died on July 21, at the age of 86.

Sir David Orme Masson (1858–1937) was a son of the Regius Professor of English in the University of Edinburgh. He was a student at the Edinburgh Academy, and afterwards graduated at Edinburgh University. After a short period of research under Crum Brown he became assistant in 1880 to Sir William Ramsay at Bristol. In 1886 he became Professor of Chemistry in the University of Melbourne. A brilliant and inspiring teacher, Masson built up a fine school of chemical research, and thereby exerted a steady influence upon the development of Australian science. He was a leader in the foundation of Australia's scientific institutions. Among these were the Commonwealth Advisory Council of Science and Industry, of which he was deputy-chairman from 1916 to 1920; the Australian National Research Council, of which he was president from 1922 to 1926, and the Australian Chemical Institute, of which he was in 1924 the first president.

Masson's researches were concerned chiefly with physical chemistry, more particularly with the theory of solutions, ionization and chemical dynamics. He had remarkable originality and foresight, so that a long series of important papers came from his laboratory. He was a keen exponent of the ionic theory in its early days, and made many contributions to it. He was the author of important and prescient suggestions respecting the periodic system, the composition of the atom and the laws of electrolysis.

He was a man of great charm, and had a rare gift of lucid exposition. In his long life in Australia he grew to be a loved and venerated leader. He became a Fellow of the Society in 1903.

LIONEL WALTER, second BARON ROTHSCHILD OF TRING, who died on August 27 at Tring, aged 68, was greatly devoted to zoology all his life. Before he was thirty he had become in that branch of biology an outstanding figure of world-wide reputation. The cottage he built at Tring in 1889 for the housing of his collections was the small beginning of a museum that has since grown to a great size and contains series of specimens, such as those of anthropoid apes and cassowaries, which are unequalled in any public museum. The greatest asset in the research department is the collection of some two million Lepidoptera providing invaluable material for the study of evolution. All the collections were at the service of specialists, who found the museum an ideal place for working. Lord Rothschild's writings on systematic zoology were very numerous and varied, and his activities were so much appreciated that many foreign scientific societies elected him an honorary member. In 1898 the University of Giessen conferred on him the degree of Dr.Phil. In 1899 he was elected a Trustee of the British Museum; and in 1911 he became a Fellow of the Royal Society. From 1899 to 1910 he represented Mid-Buckinghamshire in Parliament. In a codicil to his will he left his museum to the Trustees of the British Museum on condition that it be continued in some form or other for the benefit of zoological research.

ALBERT HEIM died on August 31 at the age of 88. For sixty years he had been the best known figure among Alpine geologists. His statement of the Glarus "Double Fold", a theory originating with Escher, brought him into prominence in 1878. His eventual recognition in 1902 of the truth of the rival one-way interpretation was a notable event. His "Geologie der Schweiz", 1919–22, furnished an imperishable memorial.

Heim's geological interests were very wide. He made important contributions to stratigraphy, tectonics, metamorphism, glaciology and the study of scenery. He wrote fluently, and had rare skill both as draughtsman and as engraver. With his own hand he engraved many of the illustrations that adorn his text. His delightful personality in its venerable stage will long be remembered both at home and abroad. He was the doyen of the Society's Foreign Members and the last survivor of those elected in the nineteenth century.

ALFRED JAMES EWART was a botanist of varied interests. He was born in Liverpool in 1872 and educated at Liverpool Institute and University College, Liverpool. When only twenty-two he published two contributions to plant physiology, dealing respectively with vitality and germination of seeds and the germination and growth of pollen tubes. At Leipzig under

Pfeffer, he carried out an investigation on assimilatory inhibition in chlorophyllous plants. At Birmingham he occupied a succession of posts ranging from science master at King Edward's School to Deputy-Professor of Botany in the University. During this period Ewart published a number of memoirs on various aspects of plant physiology, including a book on protoplasmic streaming in plants and a paper on the ascent of water in trees which appeared in *Philosophical Transactions*. But, the best known of his botanical achievements during this period of his life is his translation of Pfeffer's "Physiology of Plants" published by the Oxford University Press in three volumes.

In 1906, Ewart was appointed to the Chair of Botany and Plant Physiology in the University of Melbourne. Until 1921 he also held the post of Government Botanist to the Government of Victoria. In Melbourne Ewart maintained his interests in plant physiology and a number of papers dealing with researches on vitality of seeds, ascent of water and photosynthesis appeared from his pen. He wrote also on questions of forestry and mycology. Perhaps his most important publication in the sphere of systematic botany is his "Flora of Victoria".

The gap caused by the death of BARON RUTHERFORD OF NELSON, still in full vigour of mind and body, is one which will be felt far and wide for a very long time. He unravelled the tangled skein of radioactivity with a persistence and acumen rare in the history of science. He modified all our views of atomic theory, and showed that the nucleus of an atom carried most of the mass and all the positive charge. His crowning feat was the first step in the transmutation of matter. He founded schools of able enthusiasts at McGill, Manchester and Cambridge. He combined rare judgment and insight with remarkable manipulative skill and by his genius inspired others to fine achievement. Honours deservedly poured upon him: Order of Merit, Nobel Laureate, President of the Royal Society, a Peerage, but none of these could spoil his friendly, strong and simple character.

I shall not attempt on this occasion to review his scientific achievements, nor to tell the story of his life. So great and wide have been the sense of loss and the desire to pay him tribute that innumerable references have already been made to him. Friends, colleagues, students and many who had never met him but had grasped the value of his work, have recorded their admiration and drawn pictures of him from their different points of view. Already they have constructed for him an imperishable record, though indeed the marvellous advances in scientific knowledge which we owe to him have already given him honour and a name which can never be forgotten. It is only left to me to record here the veneration and affection which in this Society have been his in abundant measure.

Sir HERBERT MAXWELL was born at Edinburgh in 1845 and was educated at Eton and Oxford. He was kin to those who in earlier and doubtless easier days took, or tried to take, all knowledge for their province. In no field did he work intensively, but what he may have lost there he gained in extension, and in a resultant balance of judgment. In the event he achieved a remarkable distinction.

All his life he was a student of the country-side. The Annals of Scottish Natural History, and various horticultural Journals contain short contributions detailing his observations. He was in many ways the Gilbert White of his country and of the neighbouring shires. He was President of the Society of Antiquarians of Scotland for many years and Rhind Lecturer in Archaeology at Edinburgh University on two occasions. He was in 1910 Lecturer on Scottish History in the University of Glasgow. He wrote a number of historical and biographical pieces. For painting and sketching he had both talent and enthusiasm, and their pursuit occupied much of his leisure. He was Member for his county for 26 years, holding his seat in an uninterrupted series of seven general elections. He was a Lord of the Treasury, a Government Whip, Chairman of the Tariff Reform Party in the House of Commons (1903) and Lord Lieutenant of his County. His interest in Scottish History and Literature is reflected in his Chairmanship of the National Library of Scotland. He was an officer in the Ayrshire militia for 21 years. He was a keen fisherman and a good shot.

Diffused as were his interests, his energies were rarely misdirected. North of the Tweed he occupied a very special place in the eyes of his countrymen—a very old and wise Nestor who could give advice to the point on most matters and did not hesitate to tender it.

The sudden death of JAMES RAMSAY MACDONALD from heart failure when on a voyage to South America will be deeply regretted by the Fellows of the Society. In his early days he was a keen student of science, and would no doubt have held to research and teaching all his life had he not become attached to a remarkable group of social reformers in Bristol fifty years ago. Even when he left Bristol to come to London he continued his studies of scientific subjects privately and at Birkbeck College with a view of obtaining a scholarship or exhibition in the old Science and Art Departments examinations. It was impossible, however, for him to find time outside his daily working hours for the intensive study required, and he had to abandon his early hopes.

This is not the occasion for an account of Ramsay MacDonald's great political career. We remind ourselves, however, that when in 1935 he resigned his office as Prime Minister and First Lord of the Treasury, he became Lord President of the Council, and as such was responsible to Parliament for the reports of the Department of Scientific and Industrial Research and of the Medical and the Agricultural Research Councils. He took a particular interest in the work of these Departments. He had indeed maintained his scientific interest throughout his life, and on the ground that he "had rendered conspicuous service to the cause of science" he was elected a Fellow in 1930.

News has just been received in England of the death of Sir JAGADIS CHUNDER BOSE. He was born in 1858, and was educated at Calcutta and Cambridge. In the closing years of last century Bose made a number of ingenious experiments on Hertzian waves. He succeeded in generating waves of such high frequency that it became possible to examine their reflection, refraction and polarisation by means of instruments little more cumbrous than their optical analogues. In later years he was much interested in the response of plants to various forms of stimulus. He founded and directed the Bose Research Institute. He presided over the Indian Science Congress in 1927. He became a Fellow in 1920.

Fellows will have learnt with great pleasure that HIS MAJESTY KING GEORGE VI has graciously consented to be the Patron of the Society, thus continuing a practice which has been, from the foundation of the Royal Society, a source of pride and gratification.

We are grateful also for His Majesty's promise to continue the annual grant of two Royal Medals.

I would like to refer briefly to certain interesting events of our past year.

An informal discussion between the Officers of the Society and an Officer of the National Academy of Washington has led to a very happy result. It was proposed that in alternate years the Society should invite and entertain a distinguished lecturer from the United States, and the Academy should arrange the converse proceeding. The Pilgrim Trust was consulted on the question of providing the money required for the scheme, and most generously offered a sum of 1500 guineas, which in the opinion of the Trust should provide for suitable honoraria to be paid to the lecturers in six successive years. An exchange of communications between the Academy and the Society has resulted in the completion of the necessary arrangements, and the first Pilgrim Trust Lecture will, it is expected, be given in London in the coming summer.

Fellows will, I am sure, feel that no more agreeable way of emphasizing the cordial relations between American and British science could have been devised. Although modern communications are so rapid and complete and views spread so quickly, there is a personal character in the research of each man who breaks into a new field, and this interesting and important character can only be communicated by the man himself. In my opinion these lectures should not be mere summaries of past work, nor general discussions of scientific advance. It might be their special feature that they should transfer from one side of the Atlantic to the other new ideas which had already begun to be fruitful and promised wide expansion in the future. Such lectures would associate workers in a common task, and encourage correspondence and the formation of friendships. The choice of lecturers would not be determined on the same plan as the choice for the awards of medals or other distinctions, but would rather bring into prominence the most important lines of advance of the day. The progress of science would be the object of the Pilgrim Trust Lecture, and not the honouring of scientists.

The provision of funds for six years is sufficient to make trial of the plan. If it is successful, as we may be sure it will be, we hope that means for its continuance will be forthcoming.

I am tempted to further hopes. It may be that the universal wish to promote peaceful relations between the nations of the world may find some who are willing to follow the example of the Pilgrim Trust. Of all the enterprises of mankind the acquisition of Natural Knowledge pays least attention to the divisions of men. We have national industries, national trade, national literature, national art, national characteristics, even national religion, but there is only one Nature for us to know. One could wish that the seasonal interchange of men to show to other nations what new illumination was dawning in this or that subject of enquiry could be firmly established and honoured by the emphatic recognition of the academies and indeed of the State itself. No greater gift could be made by one people to another than an illuminating idea; it would deserve a people's welcome.

In April the delegates to the General Assembly of the International Council of Scientific Unions met for conference in our rooms. Good use was made of the opportunity to discuss some important scientific matters of an international character. The meeting was a particularly pleasant one, largely on account of the personality of the President, Dr. Nörlund, whom many of us are now happy to remember as a friend. Dr. Nörlund conveyed to the General Assembly an invitation from the Royal Danish Academy of Sciences to meet in Copenhagen in 1940, which was accepted.

Last November, I spoke of the large sum which the late Mr. H. B. Gordon Warren had bequeathed to the Society. In the course of the year two Fellows have been appointed under the terms of the bequest. It will be remembered that Mr. Warren desired that the money should be applied to the encouragement of research in metallurgy, engineering, physics and chemistry. One of the new Fellows, Mr. Hartley, proposes to work on oil drops, films and solutions, and the other, Mr. Randall, on the mechanism of fluorescence in pure and impure solids. These subjects have that leaning towards the application of science which was clearly in Mr. Warren's mind; and it is interesting to observe how easily the choice falls into accord with great industrial problems of the day. We associate the study of phosphorescence in the past with but a few enquiring minds, among whom Sir Herbert Jackson, who was so recently with us, was prominent. When C. V. Boys gave the children's Christmas Lectures on soap bubbles at the Royal Institution in 1899, it was generally felt that the subject was redeemed from ultra-childishness only by the exceeding technical skill and the ingenious explanations of the lecturer. In these days the film, and phosphorescence, are very important indeed.

By the addition of these two Warren Fellows the research "staff" of the Society is still further increased. The work which has been accomplished during the year is described in an appendix to the Council Report. Fellows will agree, I am sure, that it makes a fine record.

During the year, four "discussion meetings" have been held, with much success. An attempt has been made on each occasion to collect, even from considerable distances, all who were interested in the subject for discussion, including particularly the younger men who might be working on it. The purpose in view was the encouragement and help that would be expected to follow from personal contact and exchange of information. The Royal Society has some right to look on the young workers of the country as, in a sense, *in statu pupillari*, and has a certain responsibility for their effectiveness. The very considerable sum—more than £30,000 a year—which the Society has undertaken to devote to the improvement of Natural Knowledge gives it power, up to a point, to control the direction of research; and in doing so, to help the workers to do their best. The impression formed at the discussion meetings was certainly one of conviction that they are a help and a pleasure to those who attended them.

The Council Report contains notices of bequests and gifts increasing further the resources of the Society which are to be devoted to research. The reference to Dr. J. H. Stothert will remind Fellows that a lamentable accident on an Austrian river has deprived medical science of an enthusiastic young worker who might have made important contributions to knowledge. Like Moseley, who also was young, he has left his fortune—in his case as a reversionary interest—to the Royal Society. The Society cannot but feel a tenderness in handling such gifts as these.

On a very interesting occasion during the year, a number of letters, which had long remained under seal in the Society's safe, were opened in the presence of Fellows and others. There were no inscriptions governing the manner and time of their opening, and it seemed to the Council that any limits must long have been passed. Several of them were very interesting in their matter, quite apart from the dramatic circumstances of their disclosure so long after their authors had written and sealed them and handed them for safe keeping to the Officers of the Society. The most interesting of all was a letter of Faraday's, written in 1832; it has been published in the Society's *Occasional Notices,* 2. It shows that he was then entertaining the idea that the "diffusion" of magnetic and electric forces was comparable with the spread of waves on water or in air, and had indeed a vibratory character. Perhaps because of preoccupations which hindered the development of the idea, and because of the difficulties of experiment, it was not until 1846 that he made public reference to the matter. In the latter year, Faraday had to take Wheatstone's place on very short notice at a Friday Evening Discourse at the Royal Institution, and found that when he had completed the description of the apparatus which he and Wheatstone had arranged during the afternoon, he had still in hand a portion of the lecture hour. He then went on to speak of his "Thoughts on Ray Vibrations" which afterwards formed the subject of a paper in the *Philosophical Magazine.* It was this paper that led Maxwell (*Phil. Trans.* 1865) to put Faraday's experimental results in a mathematical form, and finally to frame the electromagnetic theory of light. Until this letter was opened at the Royal Society meeting there had been no indication that Faraday's thoughts on ray vibrations had been simmering for so long.

The Report of Council describes alterations of some importance which have been made in the Statutes.

The Report also refers to progress made in the cataloguing, arranging and binding of manuscript letters belonging to the Society. It is well known that the Library contains very valuable material which has never been fully examined. It is surprisingly rich in historical documents, and also in early books and pamphlets. As these are brought into order, the value and interest of the Library will be much increased. The work requires, however, considerable time and labour, and is therefore expensive. The funds provided by the late Sir Arthur Church are at present being devoted to this purpose.

I should like to take this opportunity of welcoming our new Assistant Secretary, Mr. Griffith Davies, who will, I hope, find interesting work with the Society for many years to come. I would like also to thank Mr. Winckworth for his past services as Assistant Secretary, and to express the general satisfaction that he will now occupy himself with the most important task of the Assistant Editor of our publications.

One more matter, which has been mentioned in the *Occasional Notices*, deserves the special attention of the Fellows. Professor Salisbury made a generous donation of £25 towards the decoration of our rooms. The example is excellent. Our rooms are not large, we wish they were much larger. If we are not to enjoy the dignity of spaciousness, we can at any rate make the rooms beautiful; and there can be no doubt that much can be done to that end.

A cursory glance over the research work described in the scientific publications of to-day shows that remarkable interest is concentrated on magnitudes which are too small to be examined in detail under the microscope and too large to be studied conveniently by X-ray methods. Such magnitudes are to be found in all lines of research, medical, industrial, and purely scientific. Their behaviour presents numerous problems of great interest, and also of considerable difficulty. Solutions are of pressing importance, because the want of knowledge is in all cases a considerable hindrance to progress. When in the course of our work we arrive at these magnitudes we realise that we are facing a key position.

The microscope makes it possible to detect objects as small as a few hundred Angstrom units in diameter, but it is far from revealing the details of objects so small as this. There are other optical methods of detecting such magnitudes. Thus Langmuir has recently shown how the polarization effects of films no more than a few dozen A.U. can be made visible: but again this method does not supply a means of examining detail.

The X-rays in a sense go too far. Their wave-lengths are such that the crystalline arrangement of atoms and molecules can be measured with very great accuracy, but their field of view is too narrow to take in the details of larger structures. Thus there is a gap in the means of enquiry, and it is remarkable how consistently the particular deficiency has inconvenient results.

Magnitudes of this order occur for example in the metallurgical field. Their importance is more obvious now that the structures of metals and their alloys are better known. The X-ray methods determine with accuracy the details of the crystal structure of iron and its alloys, but such information is insufficient for a prediction of the behaviour of a specimen of steel. As Smekal has observed there are certain properties which are clearly connected with structure, and are "insensitive" to any treatments to which the steel has been submitted in its previous history. But there are other properties, to be described as "sensitive", which can be modified profoundly by treatment, such as tensile strength, plasticity, and hardness as well as electrical and magnetic properties, and these are most important qualities in practice. Long ago the microscope showed the metal to be an assemblage of grains; and the conditions of the assemblage are clearly connected with the "sensitive" properties. But the exact details of the connexion are difficult to investigate because they fall within the region in which direct illumination fails. Metallurgical theory hovers continually over the idea that a metal or an alloy contains minute groups of atoms or is even a compound of such groups which may be called crystallites since the arrangement of the atoms within each one is perfectly regular. The X-ray diffraction is regular and the lines of a "powder diagram" are clear and sharp. Thus Gough and Wood in their examination of the fatigue of metals due to the cyclic repetition—sometimes to millions of times—of an imposed stress, found that the visible grains gradually broke up to an extent which in any one experiment depended on the magnitude of the stress. Fracture in any one region occurred when the break up into crystallites was complete. It did not imply the disruption of atom from atom resulting in complete disarray, but merely a separation into minute crystals the magnitudes of which were arranged more or less closely about some average. This was shown by the form of the X-ray photograph. A definite stage had been reached in the break up of the material. The existence of such an average would imply that the dimensions of the crystallite are in some way referable to numerical relations between the form or dimensions of the atoms of the metal: analogous to but far more complicated than the formation of the benzene ring of definite form and size from atoms of carbon each of which has tetrahedral qualities.

The discussion as to the specific existence, nature and effect of crystallites has been conducted with great eagerness, very much research on the mosaic structure of crystals in general has been undertaken, and several interesting theories have been put forward. At first theories were suggested which would have provided a super lattice, consisting of a regular arrangement of crystallites, even in the case of a pure metal. But this suggestion could not be maintained, as it evolved a second linear dimension out of a first. Buerger has suggested that the grain-like structure of a metal is due to conditions of growth, various crystalline processes meeting and joining together in irregular fashion during the formation of the whole mass. This however would lead to a casual formation which does not seem to be in accord with metallurgical experiment. G. I. Taylor's ingenious theory of the hardening of a metal by working requires the existence of crystallites of some form. The whole question is still obscure, yet it is extremely important because the properties of metals and alloys depend to a large extent on the grain-like structure which they possess. Whether so-called "crystallites" are formed under some law governing their size or are merely accidental assemblages, they are a centre of interest in the examination of metallic properties.

Similar conditions prevail in other cases where the behaviour of materials is under consideration. In April of this year the International Association for the Testing of Materials met in London. It was attended by about 800 persons, many of whom had come from abroad. The subjects dealt with centred round the use of materials for every kind of engineering design and every kind of manufacture. Under cover of a conference on testing it drew together an imposing assemblage of men engaged in the constructive work of the world. In any constructive work the testing of the material must be a decisive factor in making the design and in building to it. The design of the test itself requires the most careful consideration, because it is always a compromise between what is possible and what is practicable. Knowledge of what is possible depends on scientific research and is related to scientific problems of the greatest interest and the most varied nature. Knowledge of what is practicable is related to other interests but is also founded to a large extent on scientific research. Thus the work of the conference was closely connected with pure scientific research, depending on results already obtained and suggesting numerous opportunities for the increase of knowledge.

It was remarkable that in the case of one material after another the discussion drew attention to the importance of grain-like structure, and showed that the "grain", if I may extend the word widely from its general use in metallurgy, was the object of attack. Thus in the vast variety of fibrous materials, the fibre corresponds to the metal grain, and its study is quite as interesting and important. In all colloidal problems the condition and properties of the minute particle are fundamental. In materials derived from living organisms, the cell and its parts are the centre of interest and of course somewhere in the region of which I am speaking are the outposts of life itself. Even in dielectrics and lubricants, the groupings of atoms and molecules determine the general behaviour.

Moreover, a very considerable change in the use of materials for construction has come about in recent years in consequence of the fact that the gradual changes due to time have become really important. The so-called "creep" of materials is now one of the chief pre-occupations of the engineer. Its new importance is due to two causes. In the first place the development of machinery has necessitated more perfect fitting, and less allowance for clearance than was at one time the case, as for example in modern turbines and internal combustion engines. In such fine adjustments a creep of one part in a thousand is a very serious matter. In the second place the working temperatures have been greatly increased, and creep is thereby encouraged. There is no doubt that in any specimen but a perfect crystal slow changes take place continuously. At every moment molecules are being helped over the barriers which have kept them from positions of greater equilibrium. In this way new crystallizations are set up, or older crystallizations extended. Strain may encourage transfer from one position to another. One might almost say that every portion of a solid is a liquid for a certain fraction of its time, and that the atoms in that portion are capable of a movement which is restricted and guided by the stabilizing action of their surroundings.

The laws which govern these movements are very complicated, and detailed knowledge is scanty though badly wanted. Thus according to Dr. Bailey, a pioneer in these matters, the addition of 1 % chromium to a 0·5 % molybdenum steel increases its initial resistance to creep below a certain temperature and lessens it above. It is probable that the addition of chromium atoms locks the grain structure so long as they stay where they are: but heat facilitates their moving, all the more readily because the complicated alloy has the looser structure. Once they have moved the material would be better without them. But such a rough explanation would be well set aside for a detailed knowledge of the processes involved. Here are very interesting problems of physics and chemistry.

The careful examination of a visible cellulose fibre shows, it is said by some, that it is built up of lesser fibres, fibrillae or fibrils which again consist of ellipsoidal objects of dimensions roughly 1·5 and 1·1 μ. Each such object may contain many millions of cellulose chains, but very little is known of the structure of the contents or of the sheath that encloses them and seems to be the source of their characteristic influence. Chemical analysis and X-ray examination give a satisfactory picture of the cellulose chain-like molecule, and some information also of the details of the molecular assemblages. But information is wanted respecting the larger groups and the fibril formation on which the fibre properties obviously depend. If the fibre belongs to a living organism, change with time may be synonymous with growth. If the fibre is an element of some material in use, it is still subject to change which may seriously affect its quality.

Change may be external or internal. The slow rearrangements of recrystallization or devitrification are due to internal forces: but surface changes due to reactions with surrounding atoms such as corrosion or hydration may also affect behaviour. Naturally such surface changes are the more important the smaller the particle of the substance, as the colloid chemist points out. Thus, for example, it is a much discussed question as to how clay holds the water that is associated with it. The X-ray analysis supplies a very reasonable picture of the clay crystal; the positions of the atoms of oxygen, silicon, aluminium, magnesium, iron and the rest are known with considerable accuracy. But the remarkable properties of clay are dependent on the behaviour of the larger flake-like assemblages of colloidal dimensions, which lie between the direct observation of the X-ray methods and those of the microscope.

In dielectrics the slow changes of time bring about rearrangements, hastened by the electrical tensions to which the material is subjected. The electrical forces look for the weakest point for a break-through, just as a stress discovers the weakest point of a chain or any member of a structure. Changes are therefore important. One would wish that a structure was like the "Deacon's shay" which was so designed that every part was as strong as every other so that when the shay came to its end, it became a heap of dust upon the road. Unfortunately that is not the case with any material in use: and whatever its structure an equal balancing is apt to be destroyed by changes in its grain-like condition.

Perhaps the structure of the huge protein molecules may suggest a way of closing the gap in our knowledge and our means of enquiry. It is a very striking fact that their magnitudes tend definitely to group themselves about certain values, which moreover are simply related to one another. They are not mere groups of atoms thrown together without design. Their definite formation implies obedience to rules which must be in force at the beginning of the assembling, and are in force until an unavoidable result is reached. This would mean, as indeed a vast number of observations already imply, that the junction of carbon atoms is governed by strict geometrical laws of distance and orientation. It has indeed been pointed out by Dr. Wrinch and others that the long chains consisting of two carbons and one nitrogen in regular succession can be formed, under the guidance of the rules mentioned, into space-enclosing sheets presenting an external appearance of linked hexagons, and the number of sizes to which these assemblages can attain is limited. Possibly we have here an example of a form of procedure from the groupings of a few atoms to the larger assemblages of thousands, the process depending on a certain obedience to laws of building which have been shown to hold in the simpler case. We are encouraged to hope that this may be so, by the unexpected strictness and definiteness of the building rules in the cases which fall within the scope of the X-ray methods.

The constitution of the solid body is being examined now as it has never been possible to examine it before. We are not surprised that it is found to possess a grain-like structure, nor that this structure is of first rate importance. It is not only of interest from the purely scientific point of view, but it turns out to be of fundamental importance to all the constructive work of industry and to all the examinations of living constructions within the domain of biology. In the effort to know its details and to understand their significance a host of interesting scientific enquiries make their appearance, so that industry and science more than ever play into each other's hands. It is certainly to be expected that from these tempting labours there will result much improvement of natural knowledge.

Awards of Medals, 1937

Sir Henry Dale is awarded the Copley Medal. His most important contributions to physiology and pharmacology lie in two different but closely related fields: (1) the isolation of certain chemical substances, notably histamine and acetylcholine, from animal tissues, and (2) the discovery of the part played by these in a large number of important physiological and pathological processes.

His earlier work (1905–11) on the active principles of ergot led to progress in many allied subjects. The study of histamine, isolated from ergot extract and later found as a normal constituent of certain tissues, has modified profoundly our views of the capillary circulation and of the conditions known as "wound shock" and "anaphylactic shock". In 1914 he became interested in the choline esters and with extraordinary prescience singled out acetylcholine as the most interesting member of the series and pointed out the extreme likeness of its action to that of stimulating the parasympathetic.

In 1924 Loewi demonstrated that a substance indistinguishable from acetylcholine is liberated by the heart when the vagus nerve is stimulated. The researches of others, prominently among them Dale himself and his colleagues, have since shown that acetylcholine is liberated at many other junctions between conducting tissues, and the results with acetylcholine and adrenaline are embodied in the description of nerves as "adrenergic" and "cholinergic". Recently convincing evidence has been given by Dale and his collaborators that acetylcholine plays an important, possibly an essential, role in the transmission of impulse from nerve to voluntary muscle: a discovery which has direct practical bearings on muscular fatigue and in various pathological conditions, and also is of the greatest interest in the theory of the mechanism of the nervous and neuro-muscular systems.

As Director of the National Institute for Medical Research, Dale has inspired and directed a wide variety of investigations outside his special field, and numerous investigators from many countries have worked under his guidance.

A ROYAL MEDAL is awarded to Professor NEVIL VINCENT SIDGWICK. He has always been primarily interested in the causes which determine molecular structure, and his earlier experimental work chiefly dealt with such subjects as tautomerism, and the vapour pressures, boiling-points and solubilities of isomerides. The development of the conception of the nuclear atom, more particularly by Bohr and Moseley, made possible for the first time a quantitative treatment of chemical valency other than purely formal, and the first steps in this direction were taken by Langmuir, G. N. Lewis and Kossel during, or just after, the war. Others followed with theoretical or physical extensions.

Sidgwick's post-war experimental work has all been concerned with particular problems of structure, utilizing to the full available physical methods of attack. To take a few examples, he has shown the existence of co-ordination compounds of the alkali metals, and has demonstrated the co-ordinating properties of the hydrogen atom. In particular, it was he who distinguished clearly the existence of a third and very important type of chemical binding, the so-called co-ordinated covalent link.

In 1927, he published "The Electronic Theory of Valency", in which, for the first time, the most diverse structural phenomena covering the whole field of chemistry were rationally systematized. The book met with immediate and enthusiastic acceptance in all quarters. In 1928, he played a leading part at a conference held at Munich to discuss chemical binding in its relation to atomic structure. In 1931, he lectured in the United States of America. His second book, "The Covalent Link in Chemistry", which appeared in 1933, is based on the course then given and, like the first, has proved effective and stimulating to a high degree. In late years he has continued his work of fruitful interpretation in a series of remarkable contributions made to the Annual Reports of the Chemical Society, and in his recent Presidential Addresses to the same Society on the subject of Resonance Phenomena in Chemistry.

A ROYAL MEDAL is awarded to Dr. ARTHUR HENRY REGINALD BULLER. He was Professor of Botany in the University of Manitoba from 1904 to 1936. His original contributions to science are mainly in the field of Mycology and have been published in his Researches on Fungi. Six volumes of these researches have appeared, the first in 1909, the sixth in 1934, and he is now engaged in the preparation of further volumes.

These researches fall into two groups. The first comprises studies on the morphology, biophysics and physiology of the higher fungi, including the physiology of the mycelium and the organs produced on it, and especially of the production and liberation of spores. The second group deals with sex in the higher fungi, and his studies on this subject rank among the most important that have been made. Particular mention should be made of his observations on the process of diploidization in the higher fungi, and of the discovery, in conjunction with his student Craigie, of heterothallism in the rusts. This work has revolutionized our conception of the life cycle of these forms. Buller's studies have not been confined to one group of fungi, but include researches on Discomycetes and many groups of the Eu-Basidiomycetes, as well as on rusts and smuts. Reference should also be made to his "Essays on Wheat" and to his efforts which made possible the publication of the translation by his friend W. B. Grove of the monumental "Selecta Carpologia Fungorum" of the brothers Tulasne.

During his thirty-two years of occupation of the chair of Botany at Winnipeg, he has been a leading figure in Canadian botany, and was President of the Royal Society of Canada in 1927 and 1928. Since his retirement he has continued his researches in this country.

The DAVY MEDAL is awarded to Professor HANS FISCHER. During the past twenty-five years he has been continuously engaged in the study of the chemistry of the porphyrins, the bile pigment and chlorophyll. Starting from the knowledge that the porphyrin molecule was built up of pyrrole nuclei, variously substituted in the different porphyrins, Fischer developed controlled methods of degradation which extended the possibility of the identification of the pyrroles contained in any given porphyrin.

With the accurate information acquired in this manner as a basis Fischer proceeded, by bold and original synthetic work, artificially to prepare a large number of porphyrins of known structure, many of which proved to be closely related to or identical with natural products; his crowning achievement in this field was the synthesis of protoporphyrin, which, with iron, yielded haematin identical with that derivable from blood haemoglobin.

From the porphyrins Fischer turned his attention to the bile pigments and was able to explain the fundamental chemical features of their relationship to haemoglobin, thus paving the way for the biochemical work which is now proceeding in other laboratories and which promises to explain the actual mechanism of bile pigment formation in the body.

In recent years Fischer has applied his brilliant synthetic technique with outstanding success to the elucidation of the detailed structure of chlorophyll; his work in this field continues at the present time.

The BUCHANAN MEDAL is awarded to General FREDERICK FULLER RUSSELL. He graduated from Columbia College of Physicians and Surgeons in 1893, and began his career as a member of the Medical Corps, U.S. Army, in 1898, advancing through the various grades to that of colonel in 1917. He resigned in 1920. He was curator of the Army Medical Museum, Washington, D.C., from 1907 to 1913, and also instructor in bacteriology and clinical microscopy at the Army Medical School, where he performed distinguished service in developing and producing the typhoid vaccine which the Army has used with great effectiveness since that time. He was professor of pathology and bacteriology at George Washington University School of Medicine from 1909 to 1913 and for the following year lecturer in tropical medicine at the New York Post-Graduate Medical School and Hospital. From 1915 to 1917 General Russell was chief of the Board of Health laboratory in Ancon, C.Z., and during the world war was in charge of the division of infectious diseases and of the laboratory service of the surgeon-general's office, U.S. Army. From 1920 to 1923 he was director of the public health laboratory service of the International Health Board and from 1923 to September 1, 1935 he was general director of the Board. In 1919 he received the Distinguished Service Medal. Recently he was appointed lecturer on preventive medicine and hygiene and epidemiology at Harvard Medical School, Boston.

It was during the period while General Russell was director of the International Health Division of the Rockefeller Foundation that the Foundation gave such material aid towards the establishment of schools of hygiene in various European countries. They also contributed largely to the All India Institute of Hygiene in Calcutta and to the Singapore Medical School. General Russell was also responsible for establishing the yellow fever unit in West Africa. Large grants were given to the Health Section of the League of Nations, and the fellowship scheme under the International Health Division was considerably extended. The policy of the Foundation in these matters was moulded in no small degree by General Russell, whose influence on the development of public health services all over the world has been altogether remarkable.

The SYLVESTER MEDAL is awarded to Professor AUGUSTUS EDWARD HOUGH LOVE. He is most generally known as the author of the "Treatise on the Mathematical Theory of Elasticity" which has attained a universal reputation and remains the standard work of reference on this subject all over the world.

Before the first edition of the treatise was published in 1893 (almost simultaneously with the completion of the publication of Todhunter and Pearson's History of Elasticity), this branch of mathematical physics received little attention and its results were often regarded by engineers with suspicion. During the intervening years it has gradually established itself as one of the most reliable mathematical theories of continuous media and, unlike its sister science of non-viscous hydrodynamics, its results have been increasingly verified in practice. That this has come about is due in great part to the influence of Love's "Treatise", which, indeed, like Lamb's "Hydrodynamics", is far more than a mere treatise and embodies a vast amount of original work.

In other of his published work there is a great volume of research dealing not only with elasticity, but with hydrodynamics and electromagnetism. His earlier work was mostly on hydrodynamics, particularly vortex motion and wave-motion. He returned also at various times to electrical problems, especially those relating to the propagation, scattering and transmission of electric waves. His elastical investigations range over an exceedingly wide field, from the equilibrium of beams and plates of various shapes to the study of vibrations in a variety of difficult cases and to the applications of the theory of elasticity to problems connected with the Earth.

An enumeration of Love's researches, even when restricted to the more important ones, would be too long to attempt in this brief account; but two of them may be explicitly mentioned.

The first of these is the powerful and elegant theory of the support of the continents, of earth-tides and seismic waves and of the elastic stability of the earth, developed in his Adams Prize Essay of 1911, "Some Problems of Geodynamics", where appears for the first time his explanation of the seismic waves vibrating horizontally and transversely to the direction of propagation, waves to which Love's name is now universally attached.

The second is the remarkable paper on biharmonic analysis in a rectangle, published in the Proceedings of the London Mathematical Society in 1929. In this paper Love gives a complete solution of the biharmonic equation subject to given boundary conditions over the perimeter of a rectangle, thus solving at any rate the two-dimensional form of a problem which was described by Lamé as "le plus difficile peut-être de la théorie de l'élasticité", and had till then baffled mathematicians.

The HUGHES MEDAL is awarded to Professor ERNEST O. LAWRENCE, Professor of Physics in the University of California, the inventor (1932) of the cyclotron, the most important instrument of physical research since the C. T. R. Wilson expansion chamber. By its means ions are accelerated in a magnetic field and move within two half-cylinders which change electrical polarity in rhythm with the circulating ions, so that deuterons have been spirally speeded in a vacuum to velocities due to three million volts. These deuterons, impinging on beryllium, have produced neutrons and protons in great number, and some of the protons have been projected through the equivalent of forty centimetres of air. Many elements have been proved to be radioactive when thus bombarded by high-speed protons or deuterons.

Hydrogen molecular ions have been used also as bombarding elements with velocities due to five million volts. Such high-speed ions are available for developing the theory and practice of atomic disintegration, and Professor Lawrence and his co-workers are playing a leading part in this development.

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On the Mechanism of the Decomposition of Hydrogen Peroxide by Catalase

By D. Keilin, F.R.S. and E. F. Hartree, Ph.D.

The Molteno Institute, University of Cambridge

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In a previous paper (Keilin and Hartree 1936) it was shown that catalase combines reversibly not only with KCN and H_2S as was shown by Zeile and Hellström (1930) or with C_2H_5OOH (Stern 1936) but also with NaN_3, NH_2OH, N_2H_4, NaF and NO, giving corresponding spectroscopically well-defined compounds.

In this respect and in the general pattern of the absorption spectrum, catalase shows a striking resemblance to acid methaemoglobin. It differs, however, from the latter in three important properties, namely:

1—Its catalytic activity is at least 1,000,000 times greater than that of methaemoglobin.

2—Its iron does not undergo reduction in presence of $Na_2S_2O_4$, which rapidly reduces methaemoglobin to haemoglobin.

3—Its absorption spectrum is not affected by the addition of H_2O_2 which undergoes an instantaneous, almost explosive decomposition, while with methaemoglobin it forms a well-defined compound (Keilin and Hartree 1935).

Of the compounds which react with catalase, sodium azide (NaN_3) and hydroxylamine are of special interest. They strongly inhibit the catalytic activity of the enzyme but do not appreciably change its colour or the general pattern of its absorption spectrum. The formation of these compounds is only recognized by changes in the position and intensity of some of the bands. However, on addition of traces of H_2O_2 to azide- or hydroxylamine-catalase, the colour of the solution turns from greenish-brown to red and the three-banded methaemoglobin-like absorption spectrum is replaced by two strong bands at 590 and 554 mμ. This compound combines reversibly with CO, giving a derivative with the bands at 580 and 545 mμ, and reverts to the original azide-catalase only in presence of molecular oxygen, which shows that its iron is in the divalent state.

It was shown previously (Keilin and Hartree 1936) that the reduced compounds, although autoxidizable, are not oxidized by $K_3Fe(CN)_6$ in complete absence of oxygen. This point seemed at first to contradict the evidence that the iron of azide- or hydroxylamine-catalase was actually reduced by peroxide. It was found, however, that these derivatives of catalase can be reoxidized to the initial ferric compounds by some other oxidizing agents such as $KMnO_4$, $CuCl_2$, somewhat more slowly by MnO_2 and very slowly by $HgCl_2$. It can be considered therefore as adequately demonstrated that azide- and hydroxylamine-catalase are reduced by H_2O_2 and reoxidized by molecular oxygen.

This property of azide-catalase makes it an excellent if not unique reagent for the detection of H_2O_2 where no other reagent can be used, for instance, during the aerobic oxidation of sodium hyposulphite ($Na_2S_2O_4$)* to sodium bisulphite. These experiments with azide- and hydroxylamine-catalase suggest that the normal catalytic activity of catalase is also accompanied by a similar change of valency of its iron, or in other words, the catalytic decomposition of H_2O_2 is brought about by the successive reduction of the catalase iron by H_2O_2 and its reoxidation by O_2 according to the equations (1) and (2), the sum of which (equation (3)) is the catalytic decomposition of H_2O_2 into molecular oxygen and water. This reversible

$$4Fe^{\cdot\cdot\cdot} + 2H_2O_2 = 4Fe^{\cdot\cdot} + 4H^{\cdot} + 2O_2 \qquad (1)$$

$$4Fe^{\cdot\cdot} + 4H^{\cdot} + O_2 = 4Fe^{\cdot\cdot\cdot} + 2H_2O \qquad (2)$$

$$2H_2O_2 = 2H_2O + O_2 \qquad (3)$$

reduction is analogous to the reaction between ferric chloride and hydrogen peroxide, whereby the ferric ion is reduced and in the presence of $\alpha\alpha'$-dipyridyl gives rise to a pink compound. If the ferrous ion is not so fixed it becomes oxidized by molecular oxygen and all the peroxide is eventually decomposed.

The problem that arises now is whether the mechanism of the catalase reaction as shown in the above equations can be demonstrated experimentally.

As we have previously shown, the formation of an intermediate compound cannot be demonstrated spectroscopically, which indicates that the life of this compound is extremely short. Moreover, the main requirement for spectroscopic investigation is a very high concentration of enzyme, which in itself is particularly unfavourable for the detection of an intermediate compound.

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* In accordance with the recommendation of the International Union of Chemistry, we shall in future use the term "hyposulphite" in place of the original but misleading "hydrosulphite", when referring to $Na_2S_2O_4$ (Smith 1936).

On the other hand, if the catalytic decomposition of H_2O_2 requires the presence of molecular oxygen (as shown in equation (2)) for the reoxidation of the reduced compound, the reaction should be inhibited if the concentration of oxygen in the surrounding medium could be made sufficiently low. This can be achieved by having the reaction carried out with a very low concentration of catalase, which is possible owing to the very high activity of the enzyme. The volume of oxygen produced in this case by the direct reduction of catalase (equation (1)) would naturally be very small. If at the same time the reaction is carried out in an atmosphere of pure nitrogen and with vigorous shaking, in order to bring the greater part of this oxygen as rapidly as possible into the gas phase, the reaction (2) should be greatly slowed down and the catalase reaction thus inhibited. In fact, by carrying out experiments in pure N_2, H_2 or CO_2, and under conditions where there is no spontaneous decomposition of H_2O_2 due to shaking in an insufficiently cleaned glass vessel or to the presence of catalytic impurities, it has been found possible to inhibit the catalase reaction by as much as 99%.

MATERIAL AND METHODS

Catalase preparation—Catalase used in this investigation was prepared from horse liver by the method previously described for the preparation A_3 (Keilin and Hartree 1936). This preparation contained varying amounts of a yellow-brown pigment with a general absorption towards the blue end of the spectrum. By a few slight modifications of the method, catalase solutions were obtained which were completely free from this pigment. These modifications can be summarized as follows:

1—The liver is preferably kept for 1 day or more at − 10° C. before mincing.

2—Minced liver is extracted for 24 hr. at room temperature, instead of 2 hr.

3—Instead of ultrafiltration, the elution from the calcium phosphate is treated with ammonium sulphate (450 g./l.), filtered, the precipitate suspended in water and electrodialysed until completely free from sulphate.

This method gives a distinctly greenish preparation (D) which differs from A_3 in being free from labile non-haematin iron. Unlike A_3 this preparation gives no change in colour on addition of $Na_2S_2O_4$ and $\alpha\alpha'$-dipyridyl or on treatment with H_2S. The ratio between total and haematin iron in this preparation is about 1·2, while that in fresh preparations of A_3 may be as high as 20. In other properties this preparation of catalase differs very little from A_3.

Calcium phosphate—The calcium phosphate used during the preparation of catalase was prepared as follows: 150 c.c. calcium chloride solution (132 g. $CaCl_2 . 6H_2O$ per litre) is diluted to about 1600 c.c. with tap water and shaken, with 150 c.c. trisodium phosphate solution (152 g. $Na_3PO_4 . 12H_2O$ per litre). The mixture is brought to *p*H 7·4 with dilute acetic acid and the precipitate washed three or four times by decantation with large volumes of water (15–20 l.). The precipitate is finally washed with distilled water in a centrifuge. 9·1 g. calcium phosphate is obtained.

EXPERIMENTAL CONDITIONS

The experiments are carried out at room temperature in Barcroft differential manometers. The left-hand flask receives 3·3 c.c. of phosphate solution, as under the conditions which will now be specified, the decomposition of H_2O_2 in absence of catalase is negligible. The right-hand flask receives about 1 c.c. of dilute catalase solution in glass distilled water, containing between 10^{-4} and $10^{-3}\gamma$ haematin and is made up to 3 c.c. with $M/30$ phosphate solution of *p*H 6·8 or simply $M/30$ acid phosphate. This flask contains also a dangling tube which receives 0·3 c.c. of 0·3 % H_2O_2. The flasks are evacuated and washed out several times with purified nitrogen and after equilibration of the temperature the taps of the manometer are closed, the manometer is read, the dangling tube is rapidly dislodged, mixing its content with that of the flask, and the manometers are rapidly shaken and read every minute.

In order to obtain clear and repeatable results the experiments must be carried out under well-defined conditions which can be summarized as follows:

1—The flasks, dangling tubes and all other apparatus must be carefully cleaned with chromic-sulphuric acid, thoroughly rinsed in tap water and in glass distilled water.

2—The dangling tubes, which receive H_2O_2, are 14 mm. long and 7 mm. in diameter, which latter dimension ensures rapid and complete mixing of its contents with that of the flask.

3—H_2O_2 used for the experiments is Merck perhydrol diluted 100 times with glass distilled water.

4—The amount of freshly diluted catalase used for each set of experiments must be finally fixed by preliminary experiments in order to give a rate of oxygen evolution not higher than 40 c.mm./min.

5—Phosphate buffer solutions are prepared from pure analytical reagents (KH_2PO_4 and Na_2HPO_4) dissolved in glass distilled water.

6—The manometers, after mixing enzyme and substrate, are shaken at a rate of about 200 times per minute.

7—The nitrogen from a cylinder must be purified over hot copper.

RESULTS OF THE EXPERIMENTS

The results of these experiments which are summarized in fig. 1 and Table I clearly demonstrate that the removal of oxygen inhibits the catalytic activity of the enzyme dissolved in acid phosphate solution by 88–99%. For all practical purposes, we can say that in complete absence of molecular oxygen the catalytic activity of the enzyme is completely abolished. In fact, a very slight activity shown in these experiments is due only to traces of oxygen which are still present in the surrounding medium. Moreover, the incomplete inhibition (40–70%) of the same reaction at *p*H 6·8 is due

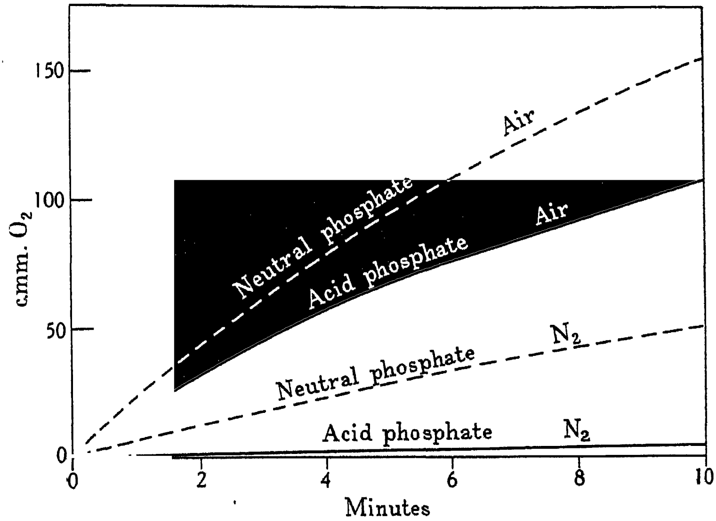

FIG. 1—Effect of pure N_2 on the decomposition of H_2O_2 by a purified catalase preparation: Catalase D (diluted to approximately 10^{-9} M with respect to haematin); 0·3 c.c. 0·3% H_2O_2; 0·03M phosphate buffer; $T = 19°$ C.

TABLE I

Exp. no.	Catalase	Phosphate buffer	O_2 evolved c.mm./min. In N_2	O_2 evolved c.mm./min. In air	% inhibition due to absence of O_2
1	A_3	*p*H 6·8	17	29	41
2	A_3	*p*H 6·8	6·5	16·5	61
3	A_3	*p*H 6·8	18	42	57
4	A_3	*p*H 6·8	3·5	11	68
5	A_3	KH_2PO_4	0·3	13	98
6	A_3	KH_2PO_4	0·5	14·5	97
7	D	KH_2PO_4	0·5	8	94
8	D	KH_2PO_4	0·3	16·5	99
9	D	KH_2PO_4	0·7	6	88
10	E	KH_2PO_4	5·0	17	71
11	F	KH_2PO_4	3·0	32	91

A_3 and D, catalase preparations previously described; E, crude extract of germinating cucumber seeds; F, crude liver extract.

to a slight non-enzymic decomposition of H_2O_2 (15 c.mm. O_2/hr.) sufficient for a partial activity of the enzyme. A similar effect is noticeable in the case of preparation E, where catalytic impurities are present.

EFFECT OF CARBON MONOXIDE

It was demonstrated spectroscopically that azide-catalase reduced by H_2O_2 combines with CO and it was shown manometrically that the decomposition of H_2O_2 by catalase is accompanied by the reduction of its iron. These results suggest the possibility that CO may act as inhibitor of the normal catalytic reaction.

Califano (1934), using a manometric method, claimed that the decomposition of H_2O_2 by catalase in CO was strongly inhibited when compared with the reaction taking place in N_2 and that this inhibition was much reduced or completely abolished by light. These results, however, were not confirmed by other authors.

Our results with the reaction carried out in purified N_2 indicate the possibility of some confusion in the work of previous authors. In fact, inhibition by CO may be due either to the absence of O_2, CO acting only as an inert gas, or to its combination with the divalent iron of reduced catalase. Thus, the former inhibition would occur only in purified CO and would not be reversed by light, whereas the latter would occur in a CO : O_2 mixture and would be affected by light. We have now shown that under conditions where purified N_2 strongly, if not completely, inhibits the catalase reaction, purified CO exerts a similar non light-sensitive effect (Table II).

TABLE II

Exp. no.	Catalase	Buffer	O_2 evolved c.mm./min.		
			CO (light)	CO (dark)	Air
1	A_3	KH_2PO_4	0	0	22
2	D	KH_2PO_4	0	1	14
3	From blood	KH_2PO_4	0	0	13
4	From blood	KH_2PO_4	—	0	14

In these experiments the inhibition due to the absence of oxygen is even greater than in the previous experiments using pure nitrogen. It can be supposed that, in absence of oxygen, there is no competition for the reduced catalase and consequently the complete inhibition observed is the sum of the "nitrogen effect" and a "CO effect". On the other hand, the experiments carried out in non-purified CO containing about 0·3% O_2, or in a gas mixture CO : O_2 = 4 : 1, using the reaction in air as a standard, did not give consistent results. It can be stated however that the purest samples of catalase are not inhibited by CO in the dark in presence of a small concentration of O_2. This shows that the reduced catalase has a much greater affinity for oxygen than for CO.

However, during the preparation of catalase A_3, fractions have frequently been obtained showing a variable but very distinct light sensitive inhibition by CO (Table III). Unfortunately, such preparations could not be obtained at will and the sensitivity of the catalase to CO in such preparations was probably due to some impurities affecting the relative affinities of the reduced catalase for CO and O_2. This supposition is supported by the fact that in some crude liver extracts CO inhibits the catalytic reaction by 40–50% (F, Table III).

Table III

Exp. no.	Catalase	Buffer	c.mm. O_2 evolved per min.			
			CO.L.	CO.D.	$CO/O_2 = 4$	Air
1	A_3	KH_2PO_4	—	—	8	16
2	A_3	KH_2PO_4	—	—	12	13
3	A_3	KH_2PO_4	—	—	5·5	9
4	A_3	KH_2PO_4	14	6	—	11
5	A_3	KH_2PO_4	10	6	—	11
6	A_3	KH_2PO_4	25	16	—	26
7	E	KH_2PO_4	—	—	12·5	17
8	F	KH_2PO_4	—	—	14	26

CO.L. and CO.D. non-purified CO containing about 0·3 % O_2 in light and dark; E, crude extract of germinating cucumber seeds; F, crude extract of horse liver.

Moreover, preparations of catalase D, which are never affected by CO, can be made sensitive to this gas in the dark by adding traces of various substances such as sodium azide, cysteine and glutathione. The inhibition of the catalase reaction produced by CO under these conditions is completely reversible in light (figs. 2, 3).

The radiation used in these experiments was the visible region of a mercury-vapour lamp, which was shown to be without effect on the catalase reaction in gases other than CO.

Summary and conclusions

1—A new explanation of the mechanism of the decomposition of H_2O_2 by catalase is proposed. According to this explanation the reaction is brought about by the successive reduction of the catalase iron by H_2O_2 and its

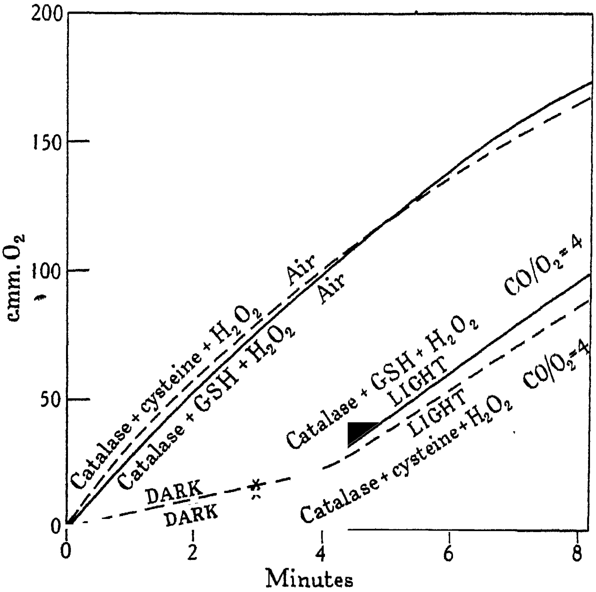

FIG. 2—Effect of CO in dark and light on catalase D (approx. 10^{-9}M haematin) in presence of 10^{-4}M glutathione and 10^{-4}M cysteine; 0·03M KH_2PO_4; $T = 19°$ C.

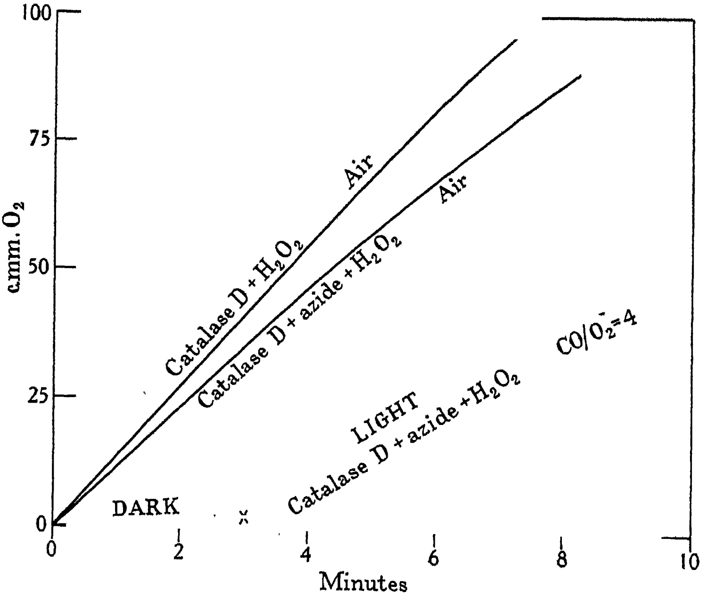

FIG. 3—Effect of CO in dark and light on catalase D (10^{-9}M haematin) in presence of sodium azide (10^{-7}M); 0·03M KH_2PO_4; $T = 19°$ C.

reoxidation by molecular oxygen; the reaction proceeding according to the following equations:

$$\begin{array}{c} 4Fe^{\cdot\cdot\cdot} + 2H_2O_2 = 4Fe^{\cdot\cdot} + 4H^{\cdot} + 2O_2 \\ 4Fe^{\cdot\cdot} + 4H^{\cdot} + O_2 = 4Fe^{\cdot\cdot\cdot} + 2H_2O \\ \hline 2H_2O_2 = 2H_2O + O_2 \end{array}$$

2—This explanation is supported by the following experiments:

a—It has been shown manometrically that the decomposition of H_2O_2 by catalase does not proceed in absence of molecular oxygen.

b—It has been shown spectroscopically with azide- and hydroxylamine-catalase that H_2O_2 is the only known substance capable of reducing catalase iron, which can then be readily reoxidized by molecular oxygen.

3—The inhibitors of catalase can be separated into two groups, those like KCN, H_2S, C_2H_5OOH which inhibit the reduction of catalase iron by H_2O_2 and those like azide, hydroxylamine and hydrazine which inhibit the reoxidation of the reduced catalase by molecular oxygen (Keilin and Hartree, 1936).

4—The decomposition of H_2O_2 by pure catalase preparations is not inhibited by CO in presence of even a very small concentration of oxygen, which shows that the reduced catalase has a much greater affinity for oxygen than for CO.

5—Some catalase preparations have been obtained which show a more or less marked light sensitive inhibition by CO. Pure catalase preparations become sensitive to CO in presence of a very small concentration of azide, cysteine or glutathione, substances which apparently inhibit the reoxidation of reduced catalase.

References

Califano, L. 1934 *Naturwissenschaften*, **22**, 249–50.
Keilin, D. and Hartree, E. F. 1935 *Proc. Roy. Soc.* B, **117**, 1–15.
— — 1936 *Proc. Roy. Soc.* B, **121**, 173–91.
Smith, C. 1936 *J. Chem. Soc.* pp. 1067–78.
Stern, K. 1936 *J. Biol. Chem.* **114**, 473–94.
Zeile, K. and Hellström, H. 1930 *Hoppe-Seyl. Z.* **192**, 171–92.

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The Monaural Threshold: Effect of a Subliminal Contralateral Stimulus

By J. W. Hughes, B.Sc.

From the Physiology Institute, Cardiff

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Introduction

It has been assumed by some workers that the intensity of a note of any frequency in either ear at the binaural threshold is one-half of the threshold intensity in either ear when used alone, i.e. at the monaural threshold. Another form of the assumption is the statement that the monaural threshold intensity is 3·0 db. above the intensity in either ear at the binaural threshold. In the course of the investigation of the possibility of forming a loudness scale, Churcher (1935), and Fletcher and Munson (1933), have found that the intensity of a note when heard binaurally may be assumed to be one-half of that of the same note heard monaurally when the note is judged to be equally loud under the two conditions of listening. In the present work, this fact has been further verified. The measurements were extended to the cases in which the subliminal note presented to the one ear was maintained at various levels below the monaural threshold, and the intensity of the note applied to the other ear to reach the "binaural" threshold was determined. In this way it was possible to find the relation between the energy required at the monaural threshold and the total energy required at the "binaural" threshold determined under these conditions. The experiments were also carried out with notes of different frequencies in the two ears in an endeavour to investigate the variations of total energy in such cases.

The apparatus

Two heterodyne oscillators were used to provide pure notes of the various frequencies used during the work. The first of these (oscillator A) was already available, and has been described elsewhere (Shaxby and Gage 1932); the second (oscillator B) was a commercial oscillator of similar frequency range, viz. 30–10,000 c./sec. *A*, if allowed to run for about an hour, was very stable in frequency, varying by only 1 or 2 c./sec. during the next 7 hr. *B* drifted for about 2 hr. fairly rapidly, and thereafter drifted quite steadily at the rate of 10–15 c./sec. every hour. *A* was therefore used as the frequency standard. It was adjusted to read correctly by the "zero-beat" method after the "settling down" period, and set to the particular frequency required; *B* was brought into unison with it. The actual frequency of *B* could be determined in this way at any time, and its drift checked.

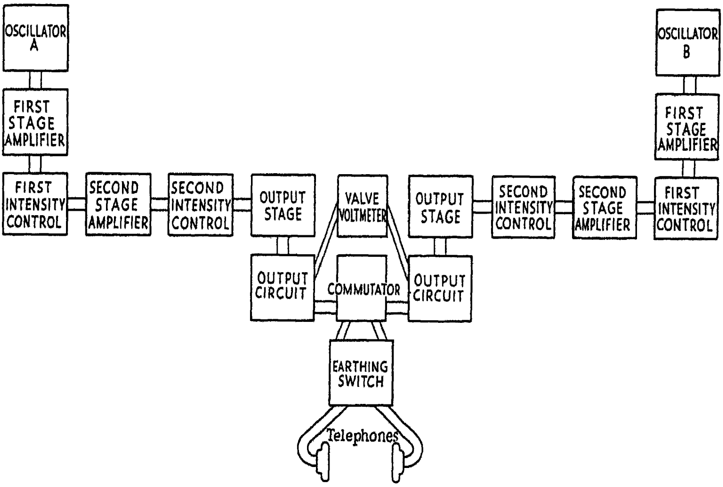

Fig. 1—Schematic diagram of the apparatus.

A schematic diagram of the apparatus is given in fig. 1, and a circuit diagram in fig. 2. It consists of two exactly similar circuits, in each of which the oscillator was resistance-capacity coupled to a three-stage amplifier with the same type of coupling. The output from the last valve was shunt-fed into a step-up transformer, ratio 7 : 1, with its secondary centre-tapped. One half of the secondary was loaded with the output circuit, consisting of a 1-megohm resistance in series with a telephone earpiece and its shunt, a resistance box variable from 0–10,000 ohms. The other half was used to drive a valve voltmeter for checking the output voltage. The latter might be made as much as 50 V (R.M.S.).

The intensity of the note given by either telephone could be controlled in three ways. (*a*) The second stage of each amplifier was fed from the first through a potentiometer of resistance 316,000ω, shunt-fed from the first stage. This resistance was suitably tapped and mounted so as to give intensity variations in steps of 5 db. at the grid of the next valve, and was used as a coarse control of intensity. (*b*) The third stage was fed from the second in the same way, using a commercial 50,000ω wire-wound potentiometer, and giving a fine control of intensity. A dial, calibrated in degrees, was mounted on the shaft of this potentiometer, and the control was calibrated in decibels by means of a Wheatstone bridge. The calibration was checked by using an electrostatic voltmeter as shown (Gage 1934) in Fig. 3 to eliminate the effect of any resistance at the sliding contact. The two calibration curves are parallel but not coincident, though the results are unaffected since they depend entirely upon the differences between two readings taken on either curve. (*c*) The resistance box in parallel with the

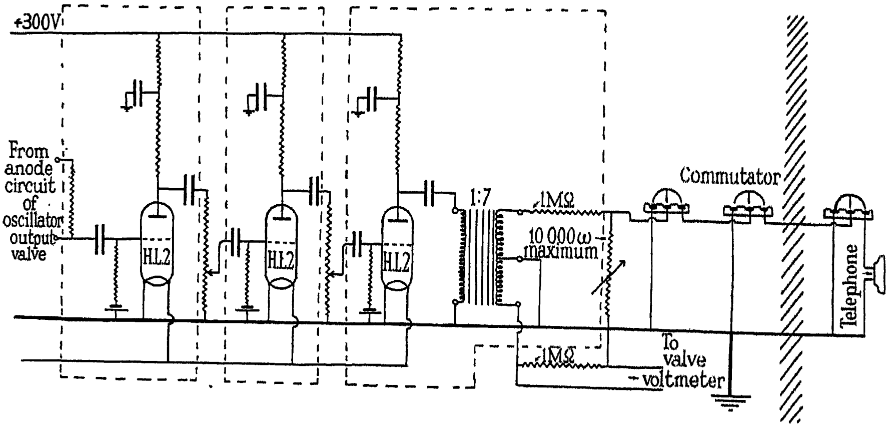

FIG. 2—Diagram of amplifier and output circuit for the telephone on the left ear. (The circuit for the other ear is exactly the same.)

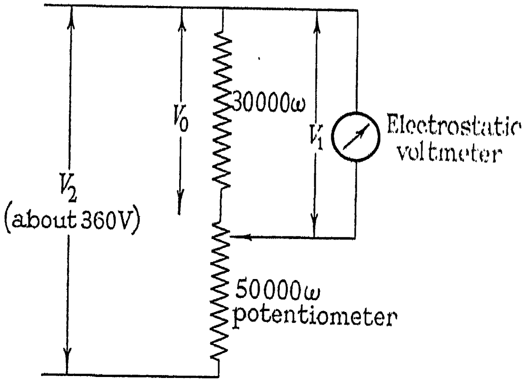

FIG. 3—Calibration of second intensity control. Decrease in db. (from 0°) = $20(\log_{10}\overline{V_2 - V_0} - \log_{10}\overline{V_2 - V_1})$.

telephone can also be used to vary the intensity. The maximum change possible is 10,000ω, so that the output load varies only about 1 % and the rest of the circuit is not affected by the variation.

In each telephone circuit there is a commutator, which short-circuits both telephones simultaneously about once a second, giving a series of tones lasting about half a second, followed by silence for half a second, and so on. In the earlier work a mercury commutator with rotating vanes was used to produce this effect, but was later replaced by a double-pole double-throw mercury switch arranged to rock once a second by an eccentric mounted on a rotating shaft. Both types of commutator were driven by a small synchronous motor through a step-down transformer from the 50 c./sec. A.C. mains. It was possible to make either telephone sound alone, with the other silent, either by including a separate earthing switch in each circuit, or by earthing, in the appropriate amplifier, the tapping point on the first potentiometer referred to above. With the maximum intensity in one telephone (not applied to the ear), no sound could be detected in the other when silenced in the latter way, showing that no "pick-up" was taking place in the wiring.

All the work described was carried out in a "sound-proof" room. Although it did not exclude altogether the noise of the heavier traffic passing the building, very little of frequencies above 100 c./sec. entered, and that of lower frequencies was only intermittent. Provided that silence was maintained immediately outside the room (and this was always the case) it proved quite satisfactory.

EXPERIMENTAL PROCEDURE

The broad outline of the experimental procedure is as follows: The two oscillators were set to the frequencies previously chosen, and the threshold intensity for one note in the right ear (with the other phone silent) was determined by a method described later. The threshold of the other note in the left ear was then determined in a similar way. The intensity of this second note was then further reduced by a predetermined amount, and the threshold of the first in the right ear redetermined with the second (subliminal) note still presented to the left ear. Immediately after the determination had been made the notes were sounded separately, without any change of intensity, to verify that neither was audible in the absence of the other. The experiment was repeated with three or four different reductions of intensity of the second note, the whole process taking about 1 hr. to complete. The output of each amplifier was then checked by the valve voltmeter, and the frequency drift noted and corrected. Ten or more of these experiments were made at each of various pairs of frequencies, and the results tabulated.

In experiments of this nature, it is necessary to be able to determine the thresholds fairly accurately, since the reductions produced by the presence of the subliminal note are of the order of a few decibels only. Various methods were therefore tried. Using a continuous note of steadily decreasing intensity was found to yield threshold settings depending mainly on the rate of decrease in intensity from about 5 db. above the final setting. Increasing the intensity of a note initially below threshold gave similar results, and also indicated that the observer was delaying his signals until he was absolutely certain that the note was heard. The method finally adopted was to use the fine control of intensity in steps of 10° on the dial, reducing the intensity a step at a time, and finding the readings at which the note was just audible, and just inaudible. For example, it might be audible at 100°, and inaudible at 110°; the threshold setting would then be taken as 105°. Extended tests of this method gave a standard deviation of the order of ±0·5 db. for sets of ten readings taken in this way. The use of a note interrupted as already described made it a good deal easier for the observer to make his decisions as to its audibility, though control tests indicated that the final settings were not significantly different from those made with a continuous note.

In practice the note used was reduced from an intensity at which it was very definitely audible. The rough adjustment was first used, with the fine control at 0° and a suitable value of shunt resistance for the telephone. Then the fine control and the variable shunt were used in conjunction with one another to arrange that the threshold setting was at a convenient part of the scale, about 3 db. below the intensity corresponding to a setting of 0°. When the two monaural thresholds had been determined in this way, the second note was reduced in intensity by the required amount, using the calibration curve of the appropriate dial, and the threshold of the first determined, starting at a dial reading of 0°. At this reading a definite note, about 3 db. above monaural threshold, would be heard in the right ear. The intensity was then reduced by means of the dial until the right-ear threshold was again reached.

The observer in the sound-proof room could communicate with the assistant operating the dials outside by means of a key which rang a bell outside the room and lit a lamp inside. The assistant could also communicate with the observer by a key which also operated the bell and lamp, in order to let him know when the threshold setting had been made. The observer signalled whether he required an increase or decrease in intensity, but was unaware of the amount of change actually made, as the assistant himself decided how many steps of 10° went to make up the change. In each series of experiments three or four different reductions in intensity of the second note were employed, and the order of these was chosen by the assistant and was unknown to the observer. In this way one avoids any tendency on the part of the observer to make his settings conform with his expectations. The observer was able to short-circuit both phones by means of a double-pole double-throw mercury switch inside the room; this was used to differentiate between the note itself and the subjective notes which sometimes appeared after a long period of listening. In practice it was found better to leave the phones short-circuited most of the time, and to listen just long enough to tell whether the note was audible or not. Occasional tests were made to see if the monaural thresholds varied during an experiment, but no significant changes could be detected. In fact, monaural threshold settings at fixed frequencies rarely varied during the day, though a rise or fall of 5 db. or so was not uncommon from one day to another. These changes are due to variations in the observer, since variations in the output voltage at standard settings of the intensity controls could be detected by the valve voltmeter; apparent changes in threshold intensity due to output variations could thus be distinguished from true threshold variations.

RESULTS

In nearly all the experiments, with exceptions indicated later, oscillator *A* and its amplifier were used to actuate a telephone on the left ear, and oscillator *B* and amplifier actuated that on the right ear. As already explained, the fixed reductions in intensity were made in the left ear, and the reductions experimentally determined in the right ear. Three sets of results taken with the latter conditions reversed, i.e. the variable subliminal note in the left ear, showed no significant difference from the results found with the same pairs of frequencies, using the normal procedure. All experiments here tabulated were carried out by the same observer.

The first sets of results were taken with notes of frequencies in the neighbourhood of 1·000 c./sec., and not differing very greatly from one another in frequency. Consider first the results at unison, 1000 c./sec., set out in full in Table I. The experimentally determined reductions in threshold intensity in the right ear for a note 3·0, 4·0 and 5·0 db. below monaural threshold intensity stimulating the left ear are shown for eleven experiments. Each single experiment gives a horizontal line in the table, and the order in which the results were taken is indicated in each case. The mean reduction in threshold intensity and its standard deviation are also given.

Now suppose the energy required by one ear at the monaural threshold is E_0. If the energy actually supplied is E_1, at an energy level x db. below E_0, then we have, by definition of x,

$$x = 10 \log_{10}(E_0/E_1),$$

or rewriting, $$E_1/E_0 = 10^{-x/10},$$

which may be written as

$$\tfrac{1}{10} \times 10^{(1-x/10)}, \quad \tfrac{1}{100} \times 10^{(2-x/10)}, \quad \text{etc.}$$

Using these latter forms of the result, it is a simple matter to calculate the energy ratios E_1/E_0 corresponding to threshold intensity reductions of x db. In this way the total energy fed into the two ears has been calculated for the three sets of experiments; the result is given below the mean reductions in Table I, and appears to show that the total energy required at both ears for the binaural threshold when a subliminal tone is present in one of the ears is equal to that required in either ear at the monaural threshold. This is a more general form of the assumption that the intensity required

TABLE I—REDUCTIONS FROM MONAURAL THRESHOLD INTENSITY IN THE RIGHT EAR

Notes of 1000 c./sec. in each ear

Number of exp.	Db. below monaural threshold in left ear			Order of presentation
	3·0	4·0	5·0	
	Db. below monaural threshold in right ear			
1	2·8	2·2	1·5	3, 2, 1
2	2·8	2·8	2·2	1, 3, 2
3	2·8	2·2	2·2	1, 2, 3
4	2·8	2·2	1·5	3, 1, 2
5	2·8	2·2	1·5	1, 3, 2
6	3·4	2·8	2·2	1, 2, 3
7	3·4	3·4	2·8	3, 1, 2
8	4·1	4·1	2·2	3, 2, 1
9	3·4	2·8	2·2	1, 2, 3
10	3·4	2·8	2·2	3, 2, 1
11	3·4	2·8	2·8	1, 3, 2
Mean reduction	$3·1 \pm 0·4$	$2·7 \pm 0·6$	$2·1 \pm 0·5$	
$(E_1/E_0)_L + (E_1/E_0)_R$	0·99	0·94	0·94	

in each ear at the binaural threshold is one-half the intensity required at the monaural threshold for either ear; the latter assumption has of course been verified in the present experiments with the 3·0 db. reduction from monaural threshold in one ear. The "theoretical" readings corresponding to columns 2, 3 and 4, i.e. the decibels below threshold for the right ear which would give a value of unity to the expression $(E_1/E_0)_L + (E_1/E_0)_R$, are 3·0, 2·2 and 1·7.

Further experiments were then undertaken to investigate any modification of the effect which might occur when the frequencies of the notes used were made to differ by various amounts. The first set was taken with two notes differing in frequency by about 15 c./sec., that in the left ear having a frequency of 1000 c./sec. The frequency difference was adjusted by feeding the valve voltmeter from both amplifiers simultaneously, and observing the needle beats, as the use of oscillator *A* for standardizing *B* had not been considered at this stage. The mean settings are shown in the first line of Table II. They indicate that the summation effect is present with no significant modification when the frequencies of the notes presented to the two ears differ by the amount mentioned.

Several sets of experiments were next carried out with a note of frequency 1100 c./sec. in the left ear, and notes of various frequencies from 700 to 1500 c./sec. in the right ear. The frequency of 1100 c./sec. was chosen so that the other oscillator could be set to frequencies of round hundreds without risking the possibility of common overtones within the range of audible frequencies. The results, set out in the table, again show reductions of monaural threshold intensities with changing frequencies of the same magnitude as in the case of unison.

After each setting of this binaural threshold in the foregoing experiments, the observer recorded his estimate of the direction from which the sound appeared to come. No very great accuracy was expected; the indications made were "Right", "Left", "Central", and in some cases "Between left and centre", or "Between right and centre". When settings of the binaural threshold were made at unison, with the constant subliminal note 3·0 db. below monaural threshold, some tendency to central localization was expected, since the two notes would then be of approximately equal intensity, but in only two of the eleven settings was any such tendency found. At all other frequencies the note was always located on the side of the ear in which the intensity was being reduced from audibility. To verify this conclusion, the procedure was reversed, so that the latter note was heard in the left ear, and the constant subliminal note in the right ear. Three sets of results were taken and are shown in Table III; owing to a mistake in reading a calibration curve, the subliminal note was not exactly 3·0, 4·0 and 5·0 db. from monaural threshold. Localization in all cases was to the left, i.e. as before on the side of the note originally audible, and the intensity reductions found give "total energies" about the same as those found for the other settings

TABLE II—REDUCTIONS FROM MONAURAL THRESHOLD INTENSITY IN THE RIGHT EAR, AND CORRESPONDING VALUES OF $(E_1/E_0)_L + (E_1/E_0)_R$

Frequencies of notes (c./sec.)		Db. below monaural threshold in left ear			
		3·0	4·0	5·0	6·0
Right ear	Left ear	Db. below monaural threshold in right ear			
1000 ± 15	1000	3·1 ± 0·6	2·2 ± 0·6	1·5 ± 0·6	—
		0·99	1·00	1·04	—
700	1100	3·0 ± 0·4	2·4 ± 0·5	1·9 ± 0·5	—
		1·00	0·98	0·97	—
800	1100	3·1 ± 0·4	2·5 ± 0·6	2·0 ± 0·6	—
		0·99	0·96	0·95	—
900	1100	2·1 ± 0·5	1·3 ± 0·3	0·8 ± 0·4	—
		1·13	1·14	1·14	—
900	1100	3·0 ± 0·3	2·5 ± 0·3	2·0 ± 0·6	—
		1·00	0·96	0·95	—
1000	1100	2·5 ± 0·6	1·8 ± 0·6	1·4 ± 0·8	—
		1·06	1·06	1·04	—
1000	1100	2·9 ± 0·7	2·3 ± 0·5	1·7 ± 0·4	—
		1·01	0·99	1·00	—
1300	1100	3·0 ± 0·5	2·4 ± 0·4	1·9 ± 0·5	—
		1·00	0·98	0·97	—
1400	1100	3·0 ± 0·5	2·3 ± 0·4	1·6 ± 0·4	—
		1·00	0·99	1·01	—
1500	1100	3·0 ± 0·5	2·5 ± 0·4	1·9 ± 0·5	—
		1·00	0·96	0·97	—
700	900	2·6 ± 0·3	1·9 ± 0·4	1·3 ± 0·2	—
		1·05	1·05	1·06	—
310	390	2·8 ± 0·0	2·2 ± 0·2	1·7 ± 0·3	1·1 ± 0·3
		1·03	1·00	1·00	1·03
310	1100	2·8 ± 0·0	2·3 ± 0·4	1·8 ± 0·4	1·4 ± 0·3
		1·03	0·99	0·98	0·97
10,000	1100	2·7 ± 0·2	2·2 ± 0·4	1·7 ± 0·3	1·3 ± 0·4
		1·04	1·00	1·00	0·99
5000	310	2·8 ± 0·0	2·1 ± 0·0	1·6 ± 0·2	1·1 ± 0·2
		1·03	1·02	1·01	1·03
Average for all frequencies		2·82	2·19	1·65	1·2
		1·03	1·00	1·00	1·01

TABLE III—REDUCTIONS FROM MONAURAL THRESHOLD INTENSITY IN THE LEFT EAR, AND CORRESPONDING VALUES OF $(E_1/E_0)_R + (E_1/E_0)_L$

Note of frequency 1100 c./sec. in left ear

Frequency of note (c./sec.) in right ear	Db. below monaural threshold in right ear		
	2·3	3·3	4·3
	Db. below monaural threshold in left ear		
800	3·1 ± 0·0	2·7 ± 0·3	2·2 ± 0·2
	1·08	1·01	0·97
1000	3·2 ± 0·0	2·7 ± 0·3	2·2 ± 0·3
	1·07	1·01	0·97
1400	3·2 ± 0·3	2·6 ± 0·1	2·1 ± 0·1
	1·07	1·02	0·99

at the same frequencies; these are discussed later. It seems that localization when finding the binaural threshold with a subliminal note of constant intensity in one ear tends to be in the other ear. This is probably due to the experimental conditions; at low intensities localization is very difficult, and the observer's judgement is influenced by the fact that the only note that can be definitely located during a setting of the binaural threshold is that heard at the start, when it is about 3 db. above monaural threshold. As the intensity is reduced to the new threshold, the difficulty of localization increases, and the tendency is to "locate" it still in the first position.

Further sets of results were next taken at 700 c./sec. and 900 c./sec. and then at rather lower frequencies, 310 and 390 c./sec.; the latter were chosen to avoid the possibility of audible common overtones. The results, shown in Table II, are similar to those previously obtained; settings were also made in the latter case with the constant subliminal note 6·0 db. below monaural threshold.

For all the results taken the ratio E_1/E_0 was found for each ear, and as the table shows the sum of the appropriate pairs of ratios is not very different from unity, as was found for the results when the two notes were in unison. The relation appears to be followed even in the cases in which the monaural threshold intensities in the two ears differ considerably.

In order to check the foregoing conclusion, experiments were carried out with frequencies of 310 and 1100 c./sec., and with frequencies of 10,000 and 1100 c./sec.; the threshold reduction of the 1100 c./sec. note was determined experimentally in each case. The relation was found to hold just as well in both sets of results, though the differences between the monaural threshold intensities at 310 and 1100 c./sec., and between those at 10,000 and 1100 c./sec. are very large, and the actual energy reaching the one ear as a constant subliminal note of frequency 10,000 or 310 c./sec. is much greater than the monaural threshold energy at 1100 c./sec. A set of results was also taken at 5000 and 310 c./sec., using the former as the constant subliminal tone, and these agreed with those previously taken. Table II gives the last three sets of results.

It was thought that, in the cases where the energy of the constant subliminal tone was very much greater than the energy at the monaural threshold of the other, the sound which was heard when the second note was reduced below its monaural threshold might have the frequency of the first note. No trace of any such sound was ever found in any of the experiments.

Two sets of results were next taken with frequencies of 310 and 1100 c./sec., but making the constant subliminal tone, viz. that at 310 c./sec., continuous after finding the threshold in the same way as before. In the first set the subliminal tone was reduced 2·0, 3·0, 4·0, 5·0 db. below monaural threshold intensity, and in the second it was reduced by 1·0, 2·0, 3·0, 4·0 db. The results, together with those obtained by the standard method, are given in Table IV. Although the first set of the two shows a "total energy" less than unity for each reduction, the second shows it greater

TABLE IV—REDUCTIONS FROM MONAURAL THRESHOLD INTENSITY OF THE "VARIABLE" SUBLIMINAL TONE AND CORRESPONDING VALUES OF $(E_1/E_0)_L + (E_1/E_0)_R$

Left ear		Right ear		Db. below monaural threshold intensity of "fixed" tone					
				1·0	2·0	3·0	4·0	5·0	6·0
Frequency (c./sec.)	Character	Frequency (c./sec.)	Character	Db. below monaural threshold intensity of "variable" tone					
310	Interrupted	1100	Interrupted	—	—	2·8 ± 0·0	2·3 ± 0·4	1·8 ± 0·4	1·4 ± 0·3
	Fixed		Variable	—	—	1·03	0·99	0·98	0·97
310	Continuous	1100	Interrupted	—	—	3·3 ± 0·7	2·8 ± 0·5	2·1 ± 0·4	1·8 ± 0·6
	Fixed		Variable	—	—	0·96	0·93	0·94	0·91
310	Continuous	1100	Interrupted	4·0 ± 0·2	3·4 ± 0·3	2·8 ± 0·3	2·2 ± 0·3	—	—
	Fixed		Variable	1·20	1·08	1·03	1·00	—	—
1100	Interrupted	310	Continuous	—	3·6 ± 0·3	3·0 ± 0·3	2·6 ± 0·4	2·2 ± 0·4	—
	Fixed		Variable	—	1·07	1·00	0·95	0·92	—
10,000	Interrupted	310	Interrupted	—	3·8 ± 0·4	3·4 ± 0·5	3·2 ± 0·7	2·5 ± 0·6	—
	Fixed		Variable	—	1·05	0·96	0·88	0·88	—
10,000	Interrupted	310	Interrupted	—	3·8 ± 0·4	3·4 ± 0·5	3·2 ± 0·7	2·5 ± 0·6	—
	Variable		Fixed	—	1·05	0·96	0·88	0·88	—

than unity, and the difference is probably not significant. In the second set, even with the constant subliminal note only 1·0 db. below monaural threshold intensity, the note heard was still that of frequency 1100 c./sec.

Another set of results was taken, using the same frequencies as above but making the 1100 c./sec. note continuous after determining the monaural threshold. Thus the note heard at the start of the experiments with the binaural threshold was continuous, and the observer recorded any change in its character that occurred as the threshold was approached. In every case a tendency to wax and wane in intensity was found, though hardly definite enough to be classed as an interruption of the note. Such a result might be expected when the 1100 c./sec. note had its intensity reduced below that of its monaural threshold, since all the preceding results indicate that it can only become audible under these circumstances when a subliminal tone is present in the other ear. In these experiments such a tone was, of course, present as an interrupted note, and the audibility of the continuous note would tend to wax and wane in the manner described. It seems probable that no definite interruption could be noticed, as the interruptions in previous experiments became less clearly defined as the threshold, whether monaural or binaural, was approached. The actual intensity reductions found are much the same as before, and are shown in Table IV.

The standard method, using an interrupted note in both ears, was employed to take two sets of results with frequencies of 10,000 and 310 c./sec. These frequencies were employed so as to work as far as possible from the region of maximum sensitivity of the ear. In the first set, the 10,000 c./sec. note was used subliminally, and in the second, the 310 c./sec. note was kept below threshold. The intensity of the 10,000 c./sec. note was too small to be audible in the observer's right ear, although it was easily heard in his left ear; accordingly the constant subliminal tone was fed into the telephone on the right ear in the second set. In both sets, for the lower intensities of the constant subliminal tone, the binaural threshold settings show a greater reduction from monaural threshold than those previously taken, as Table IV shows. When working with the note of 10,000 c./sec. some difficulty was experienced in setting the thresholds; it was hard to attain any consistency, and this may account for the differences found.

DISCUSSION OF RESULTS

The results of the above work may be summarized as follows:

If the monaural threshold energy for the right ear for a note of any frequency is E_R, and that for the left for any other frequency is E_L, then if energy $(1/n)\,E_R$ of the first frequency is supplied to the right ear and energy $(1-1/n)E_L$ of the second frequency is supplied to the left ear, a sound is just heard, i.e. a binaural threshold is reached.

This suggests that, at the threshold, the right cochlear apparatus supplies to some central mechanism* $1/n$ of some minimal energy W which is required to stimulate that mechanism, and that the left supplies the requisite deficiency $(1-1/n)\,W$ to make up the total to the necessary amount W.

Such a conception involves three assumptions, viz.:

1—that there is a linear relation between the input intensity to the ear and its output to the central mechanism,

2—that threshold intensity is the power (varying with frequency) which gives a fixed flow of energy W to the central mechanism,

3—that the outputs from the two cochleas to the central mechanism, though distinct from one another in character, are summed, as regards their power, in the central mechanism.

In other words, the auditory mechanism as a whole behaves as though it consisted of two similar microphones, and a relay connected to both. Each microphone has, at a given frequency, a linear relation between input power and the power it passes on to the relay, though the ratio of output to input in general changes with frequency. The relay draws power from both microphones, but only responds when the total power received reaches some value W. If then the input to the first microphone necessary to produce W is E_R for a given frequency, and that to the second microphone for some other frequency is E_L, an input of $(1/n)\,E_R$ to the first and $(1-1/n)\,E_L$ to the second will just give the relay the power W, and it will respond.

In a sense organ the assumption (1) of a linear relation between input and the power passed on to a central mechanism has not been proven, but the work of Adrian has shown that stimulation of a sense organ gives rise to action currents which consist of a number of periodic discharges. As the intensity of the stimulus increases, the energy transmitted in these currents increases, both by increase in the number of nerve fibres thrown into activity and by the greater frequency of discharge in each fibre. The "output power" thus increases steadily, and possibly linearly, with the intensity of stimulation.

Assumption (2) requires that the power W corresponds to a cochlear output of some constant amount independent of frequency, this amount being attained with different intensities of stimulation by notes of different frequencies. Such an interpretation is quite probable: on the theory of cochlear resonance there are separate sets of nerve fibres producing separate action currents, and it is unlikely that the efficiency, i.e. output/input ratio will be the same for these different mechanisms.

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* The terms cochlear apparatus and central mechanism are here used as "portmanteau" terms: the first probably includes nervous as well as mechanical parts of the hearing mechanism.

The third assumption is that the power outputs from the two cochleas, though differing in character, are yet summed by the central mechanism; this duality of function of a sense organ certainly occurs in vision. A mixture of red and green lights produces the sensation of yellow light, and the intensity is the sum of the intensities of the components. This is true when the "mixing" is binocular, the red light reaching one eye, the green the other. In other words, the intensity follows a simple summation $I = R + G$, while the sensation of colour as such is governed by a quite different relation, for instance, R/G.

Thus the observations recorded in this paper seem to show that two stimuli presented binaurally may result in a summation which, at the threshold, is strictly additive; expressing the intensity of each stimulus as a fraction of the appropriate monaural threshold intensity, the sum of the two fractions is unity, whatever the frequencies of the separate stimuli. The summation may be explained on the supposition that either ear, when stimulated by a pure tone, transmits to the central mechanism an amount of energy the rate of flow of which is directly proportional to the strength of the stimulus. It is supposed that the auditory threshold is reached when the rate at which energy is received reaches a fixed value which remains the same for all frequencies of stimulation; when the inflow of energy takes place simultaneously from both ears, the threshold is reached when the total power received rises to this fixed value, the contributions from the two ears adding directly, and independently of any characteristics which they may possess in virtue of the different frequencies of the stimuli giving rise to them.

It is interesting to note that an effect similar to that found in the present experiments has been remarked by Fletcher and Munson (1933) in the course of their work on loudness measurement. Their data show that "10 pure tones, which are below the threshold when sounded separately, will combine to give a tone which can be heard. When the components are all in the high pitch range and all equally loud, each component may be from 6 to 8 db. below the threshold and the combination will still be audible. When they are all in the low pitch range they may be only 2 or 3 db. below the threshold. The closeness of packing of the components also influences the threshold. For example, if the ten components are all within a 100-cycle band each one may be down 10 db." The quantitative disagreement between these and the present results is probably due to the fact that, in the former, normal binaural listening was employed, and the component tones were mixed in the telephones, so that phenomena due to resonances both in the ear and in the telephones may well have been present in addition to those due to the effect described in the present work.

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SUMMARY

The change of threshold intensity in one ear when a note of fixed subliminal intensity is sounded in the other has been determined by direct experiment. The results of the investigation show that for two notes in unison, the total energy required in the two ears at the "binaural threshold" is equal to the energy required in one ear at the monaural threshold, independently of the actual division of energy between the two ears. For two notes of different frequencies, the result still holds good in the form $(E_1/E_0)_R + (E_1/E_0)_L = 1$, where E_0 represents the monaural threshold intensity, and E_1 the intensity of the note present in the same ear at the binaural threshold, the subscripts R and L referring to the two ears. The bearing of the results on the theory of hearing is discussed.

REFERENCES

Churcher, B. G. 1935 *J. Acoust. Soc. Amer.* **6**, 216.
Fletcher, H. and Munson, W. A. 1933 *J. Acoust. Soc. Amer.* **5**, 82.
Gage, F. H. 1934 *Proc. Roy. Soc.* B, **116**, 103.
Shaxby, J. H. and Gage, F. H. 1932 *Spec. Rep. Ser. Med. Res. Coun., Lond.*, No. 166.

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The Control of Beating and of Micro-fibrillation by means of Potassium, Calcium and Sodium in the Chick Embryo Heart

Calcium Fibrillation and the Control of Beating

By P. D. F. Murray

Strangeways Research Laboratory, Cambridge

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PART I—INTRODUCTION AND METHODS

The heart of the young chick embryo, isolated in a properly balanced solution (Table I, "*PC*") of NaCl, KCl and $CaCl_2$, continues beating sometimes for over 24 hr. and always for much longer than was required for any of the experiments to be described. If, however, the medium contains a sufficient excess of potassium or calcium there appears the fibrillation described by Olivo (1924) and studied by me (1934*a*, *b*, 1935*a*, *b*), and which is identical with the micro-fibrillation observed in adult mammals and birds by Fredericq (1929, 1931). For the sake of brevity I use here the shorter term "fibrillation", indicating in the title that it is with micro-fibrillation that the paper deals.

The object of the experiments recorded in this and the succeeding paper was to elucidate further the relation between the ions and the kind of activity displayed, with special reference to (1) the effects of removing one or two of the three ions sodium, potassium and calcium, and (2) the effect upon the action of any one solution of previous treatment of the hearts with other solutions.

As the work progressed the material was changed and the method simplified. In some early experiments fragments of the auricles of 4, 5, 6 day, and even older, embryos were explanted in plasma and embryo extract clots on cover-glasses, and these were then inverted over moist chambers containing the experimental solutions. Later the use of auricular fragments was abandoned and instead the entire hearts of 2½–3 day embryos were used because these made it possible to study all parts of the heart and were more certain to beat after explantation. Finally, the plasma clots were discarded as unnecessary. Instead, the entire hearts, after being first dissected out in 0·75 % NaCl, were transferred for about an hour to the balanced saline to allow recovery from the shock of operation and to start beating, and were then placed in Jena watch-glasses containing the desired solution, after being washed by a quick passage through three watch-glasses containing the solution. The material and methods used are indicated in the text by the phrases "auricular fragments", "entire hearts", "plasma clots", "pure salines", "saline-glucose". The hearts were studied in the watch-glasses with a Leitz No. 3 (× 10) objective and × 12 ocular, a combination which revealed even the weakest fibrillation or beat. The use of varied material and methods led to no error, for all important facts were verified with entire hearts in pure saline or saline-glucose; no important differences were found. Table I shows the principal solutions used. Osmotic pressures were found by calculation, the osmotic effect of the buffer not being considered. I have never found any influence from any but very drastic changes in osmotic pressure (1935 *a*). Isosmotic NaCl was taken as

TABLE I—COMPOSITION OF SOLUTIONS, GRAMS PER CENT

Phosphate buffer except where otherwise stated in text.

Symbol of solution	NaCl	KCl	$CaCl_2$	Remarks
*PC**	0·68 or 0·75	0·06	0·03	NaCl was 0·75 % in some experiments; the alteration had no detectable effect
NaCl	0·75	—	—	—
KCl	—	0·96	—	—
$CaCl_2$	—	—	0·95	—
NaCl + KCl	0·68 or 0·75	0·06	—	= *PC* without calcium. NaCl was 0·75 % in some experiments; the alteration had no detectable effect
NaCl + $CaCl_2$	0·68 or 0·75	—	0·03	= *PC* without potassium. NaCl was 0·75 % in some experiments; the alteration had no detectable effect
Paralysing solution 1	0·53	0·26	0·023	—
Paralysing solution 2	0·51	0·285	0·023	—
Paralysing solution 1 without calcium	0·53	0·26	—	—
Paralysing solution 3	0·75	0·4	0·03	—
Paralysing solution 3 without calcium	0·75	0·4	—	—

* This was a modification of Pannett and Compton's solution. Hearts in this solution beat in a normal manner for an indefinite time.

0·75 %, KCl as 0·96 %, $CaCl_2$ as 0·95 %, glucose as 4·62 %. The balanced saline, known as *PC*, had an osmotic pressure equal to NaCl 0·75 % when its own content of NaCl was 0·68 %. Large differences of osmotic pressure were compensated by glucose; but when the difference was trivial (e.g. caused by the presence of $CaCl_2$ 0·03 % or KCl 0·06 % in one solution but not in another) it was not compensated, for I knew from many experiments that such slight differences produce no detectable effects.

In the great majority of experiments the solution contained as buffer NaH_2PO_4 0·001 % and Na_2HPO_4, $12H_2O$ 0·0286 %, to give *p*H 7·5, but when large quantities of calcium were present the buffer was $NaHCO_3$ 0·03 %, and in a few experiments, when the absence of sodium was desired, no buffer was used.

All experiments were performed at room temperature because of its convenience and because the rate at which events occur is slow enough to allow observation; at higher temperatures visual analysis would be impossible. Both kinds of material will beat at room temperature. Error due to variations in temperature was carefully eliminated, for all the more important experiments were performed on several occasions and no significance was attached (except as mentioned in the text) to any observation which could not be repeated under summer conditions.

Experimental procedures and particular solutions are indicated in the text by symbols, and those of the solutions are explained in Table I. An arrow between the symbols of two solutions indicates that the hearts were treated first with one solution and then with the other, thus NaCl → NaCl + KCl means that the hearts were transferred to the solution NaCl + KCl after previous treatment with NaCl.

PART II—THE EFFECTS OF NaCl 0·75 %, KCl 0·96 %, $CaCl_2$ 0·95 %, ALONE

Material—NaCl:	20 auricular fragments, plasma clots. Over 50 entire hearts, plasma clots. Over 150 entire hearts, pure salines.
KCl:	50 entire hearts, pure salines.
$CaCl_2$:	18 entire hearts, pure salines.

Hearts transferred from the balanced saline *PC* to NaCl 0·75 % continued beating, but the beats became progressively weaker and at the same time underwent a change of character. The simultaneity of cell contractions decreased. Finally beating ceased and the hearts were then either stationary or might show a weak fibrillation. This fibrillation was often much more active in auricular fragments of the older hearts, and in these there could be no doubt that the movement was a true micro-fibrillation, comparable to that produced by excesses of calcium or potassium in the medium.

In pure KCl or $CaCl_2$ beating was quickly stopped, though in $CaCl_2$ it might persist for as long as 3 min. In both solutions beating was succeeded by violent fibrillation of the entire heart.

PART III—THE EFFECT OF ABSENCE OF POTASSIUM OR CALCIUM

Material—*PC* → NaCl + KCl:	5 auricular fragments, plasma clots.
	13 entire hearts, plasma clots.
	131 entire hearts, pure salines.
PC → NaCl + $CaCl_2$:	16 auricular fragments, plasma clots.
	27 entire hearts, plasma clots.
	43 entire hearts, pure salines.

When the experimental solution contained potassium but lacked calcium the beat was always very greatly weakened and might stop; more usually it became restricted to the sinus or sinus and auricles. This period of depression was always succeeded by recovery, when the hearts once more displayed a weak beat continuing for hours. The times between entry into the solution and most complete depression, and from this to the most nearly complete recovery, varied greatly. Table II gives typical figures for a group of six hearts.

TABLE II

(Each vertical column refers to one heart)

Time to loss of beat in NaCl + KCl	15	21	10	10	9	17
Time from depression to recovery	15	24	18	18	16	8

Transference to a fresh bath of NaCl + KCl after recovery of the beat failed to stop it again (nine hearts), and if done during depression failed to delay recovery (three experimental hearts and three controls). This rendered untenable any attempt to explain recovery by a rise in the calcium content of the medium (e.g. by liberation of calcium from the cells).

There was never any fibrillation in NaCl + KCl; but if hearts were transferred from NaCl + KCl to NaCl fibrillation might subsequently appear.

In $NaCl + CaCl_2$, i.e. in the absence of potassium but with calcium present, beating was at first stronger and faster than in *PC*. After a time, however, the frequency and strength of beating decreased. Various disorders of beating appeared, usually about 45 min. after entry into the solution, like auriculo-ventricular block and the development of an independent pacemaker in the ventricle, and fibrillation appeared between the beats. Finally (usually after two or three hours) beating ceased while the heart continued in fibrillation. The fibrillation slowly weakened and when it finally stopped the hearts appeared to be dead. Essentially similar events occurred in the auricular fragments of older hearts.

PART IV—THE EFFECT OF VARIOUS SOLUTIONS UPON HEARTS PREVIOUSLY TREATED WITH NaCl

The experiments described in this part show that: (1) hearts transferred from NaCl to calcium-containing solutions are thrown into fibrillation, (2) this does not occur if the second solution does not contain calcium, (3) if the second solution contains both calcium and potassium the fibrillation is transient.

The experimental solutions can be divided into three groups: (A) containing calcium but not potassium, (B) containing both calcium and potassium, (C) containing potassium but not calcium.

A—$NaCl \rightarrow NaCl + CaCl_2$

Material—20 auricular fragments, plasma clots.
9 entire hearts, plasma clots.
13 entire hearts, pure salines.
26 entire hearts, pure salines, special method for which see text.

In most of the material listed the transference was carried out in the usual way and it was found that certain events, the stimulation and stoppage of the beat, occurred so rapidly that they were over before the microscope could be focussed on the heart in its new bath. In the fourth group of material (twenty-six entire hearts) therefore, the hearts were left in NaCl (1 c.c.), and to this was added 0·05 c.c. of the mixture NaCl 0·73 %, $CaCl_2$ 0·6 %; this, mixed with 1 c.c. of NaCl 0·75 % gives practically NaCl 0·75 % $CaCl_2$ 0·03 %, i.e. the solution $NaCl + CaCl_2$. The hearts were watched continuously. The duration of treatment in NaCl before the addition of calcium ranged from 22 min. to 2 hr. 28 min. The addition of calcium was followed by a strengthening of the beat, and this effect was frequently instantaneous but might be delayed for as long as a minute. It was nearly always transient, the beat stopping after from a few seconds to 3 or 4 min. In three out of eleven cases in which beating had already stopped before the calcium was added, there was a transient restoration of the beat. The behaviour of the other three groups of material was, as far as could be ascertained, similar.

Events following stoppage of the beat are described for all four groups at once because the difficulty of observation mentioned above did not arise. The collapse of the beat was followed by fibrillation. Its activity varied but was frequently great, and it was often distributed over the entire heart. It might last with decreasing activity for as long as an hour and when it had stopped the hearts appeared to be dead, having become opaque, and failing to beat again when returned to the balanced saline.

When hearts which were already fibrillating in NaCl were transferred to NaCl + $CaCl_2$, the fibrillation became more active in the second solution.

B—TRANSFERENCE FROM NaCl TO SOLUTIONS CONTAINING BOTH CALCIUM AND POTASSIUM

Material—NaCl → *PC*: 25 entire hearts, pure salines.
NaCl → paralysing solution 1: 17 entire hearts, plasma clots.
7 entire hearts, pure salines.
NaCl → paralysing solution 3: 9 entire hearts, pure salines (+glucose added to NaCl).

Of the three experimental solutions, *PC* of course tolerates beating by normal hearts for an indefinite time, but both paralysing solutions 1 and 3 contain enough KCl to stop the beating of normal hearts in about 5 min. but not enough to induce fibrillation except in the conus. The two paralysing solutions resemble one another in all their effects, but No. 3 is a better constructed solution than No. 1 because it contains the same quantities of NaCl and $CaCl_2$ as does *PC*, while in No. 1 the concentrations of these salts are lower than in *PC*.

If hearts were transferred to *PC* after short times in NaCl (roughly, less than 20 min.) the only event was a strengthening of the beat. But if immersion in NaCl were longer than this, but not too long (roughly, over an hour) a complicated series of events occurred. These events resembled those in NaCl + $CaCl_2$ after previous treatment with NaCl except (*a*) that the transient stimulation of the beat did not always occur, (*b*) the fibrillation was always transient, stopping usually about 2–5 min. after transference to *PC*, (*c*) the cessation of fibrillation was followed by beating again and this then continued indefinitely. There were sometimes two peaks of fibrillary activity separated by a period of weaker fibrillation; this double fibrillation will be discussed further in a later paper. A minor difference from the effects of NaCl + $CaCl_2$ was the often incomplete character of the stoppage of beating, a beat often surviving in the auricles although it had stopped elsewhere. The interval between the cessation of fibrillation and the return of beating ranged up to more than an hour, and was longer the longer the hearts had been in NaCl.

When the second bath was one of the paralysing solutions the beat was always stopped instantly; there was never any preceding stimulation. Stoppage was followed by transient fibrillation, and this again, in ten hearts transferred to paralysing solution 1 and in two transferred to paralysing solution 2, by a transient return of beating. This beating was nearly always weak, sometimes very weak, and was often more or less confined to the upper part of the heart. Strong beating of the whole heart was rare. Its time of occurrence is of some importance; whereas normal hearts cease beating permanently in these solutions after at most 10 min., the transient return of beating which occurs when the hearts have been previously treated with NaCl occurred later than this, for example in one group of five hearts beating was seen 13, 13, 14, 15, 17 min. respectively after entry into the paralysing solution. The final condition of hearts in either paralysing solution, whether they were normal or had been treated with NaCl, was always stationary except usually for some fibrillation in the conus.

C—TRANSFERENCE TO SOLUTIONS CONTAINING POTASSIUM BUT NOT CALCIUM

Material—NaCl → NaCl + KCl: 12 auricular fragments, plasma clots.
13 entire hearts, pure salines.

NaCl → paralysing solution 1, without calcium:
5 entire hearts, plasma clots.

NaCl → paralysing solution 3, without calcium:
8 entire hearts, pure salines + some glucose in both solutions.

The thirteen entire hearts transferred from NaCl to NaCl + KCl had been in the first solution for periods ranging from 50 min. to 1 hr. 20 min.; they were all still beating. On transference to NaCl + KCl beating was stopped either at once or within a minute, without previous stimulation, and there was no fibrillation. Thus they were stopped more rapidly than hearts which had been treated in *PC*. No return of beating was observed. The results with auricular fragments resembled those with entire hearts.

The events in the two paralysing solutions without calcium resembled those in NaCl + KCl. One observation of interest was made: the time taken to stop beating in a paralysing solution without calcium was usually from 1 to 3 min., whereas in the same solution with calcium stoppage was almost instantaneous. It should be added that in the transference *PC* → paralysing solution 3 without calcium, stoppage was, as expected, very gradual and with no return of beating.

In connexion with the experiments in this part, one accessory experiment must be mentioned. Paralysing solution 1 was made by mixing 3·5 parts of *PC* with 1 part of isotonic KCl. In order to be sure that the effects of this solution were due to the high KCl and not to the low NaCl or $CaCl_2$, four hearts were transferred from *PC* to a mixture of glucose and *PC* in the ratio of 1 : 3·5, and four other hearts to paralysing solution 1. In the paralysing solution all four hearts became paralysed, but in the mixture of *PC* and glucose all four continued beating for at least 1 hr. 40 min., after which observations were discontinued.

PART V—THE EFFECT OF CALCIUM-CONTAINING SOLUTIONS UPON HEARTS PREVIOUSLY TREATED WITH A SOLUTION CONTAINING POTASSIUM

The experiments described in this part show that hearts transferred from solutions containing potassium to solutions containing calcium do not fibrillate (except occasionally in the transference NaCl + KCl → *PC*, and of course except when there is a very high concentration of calcium in the second solution).

A—NaCl + KCl → NaCl + $CaCl_2$

Material—6 entire hearts, plasma clots.
6 entire hearts, pure salines.

The effect of NaCl + $CaCl_2$ upon hearts previously treated with NaCl + KCl was very different from that seen when the first solution was NaCl without potassium. The time of treatment in NaCl + KCl ranged from 1 to over 5 hr. In all cases the beat, if continuing in NaCl + KCl, became stronger in NaCl + $CaCl_2$, although in two it was at first stopped and then returned stronger than before. This increased strength of beating was not the mere transitory stimulation frequently seen in NaCl + $CaCl_2$ when the first solution has lacked potassium; beating continued with this increased strength. Finally, as with normal hearts in the same solution, beating ceased and was replaced by fibrillation. The time which elapsed after entry into NaCl + $CaCl_2$ before cessation of beating and onset of fibrillation was less than in normal hearts but many times longer than in hearts previously treated with NaCl alone. Thus in five hearts beating of the whole heart lasted over an hour, and in five fibrillation was still not detectable after this time. When the first solution contained no potassium (NaCl → NaCl + $CaCl_2$) beating was stimulated and stopped, or stopped without stimulation, immediately after entry into NaCl + $CaCl_2$ and fibrillation followed within a couple of minutes (see Part IV A).

Thus, the presence of potassium in the first solution protected the hearts from the instant collapse into fibrillation which occurred when the first solution contained only NaCl, and the duration of this protection shows that, in respect of sensitivity to the fibrillating action of NaCl + $CaCl_2$, the hearts had been kept in a condition comparable to that of hearts treated in *PC*. Nevertheless, since beating did not last so long after NaCl + KCl as after *PC*, and since fibrillation tended to appear sooner, the protection conferred by NaCl + KCl was less effective than that conferred by *PC*.

B—NaCl + KCl → *PC*

Material—61 entire hearts, pure salines.

The experiments just described, in which potassium in the first solution gave prolonged protection against the fibrillating action of calcium in the second, lead one to expect that fibrillation would not occur in *PC* after NaCl + KCl. This expectation was usually, but not invariably, fulfilled. Fibrillation was seen in *PC* in twelve or thirteen hearts out of the sixty-one studied; it was always transient and resembled the fibrillation seen in *PC* after NaCl except that it was usually much weaker. The behaviour of the beat in general resembled that in *PC* after NaCl. There was in at least nineteen hearts momentary stimulation, followed by stoppage or reduction of the beat from contractions of the whole heart to beats of the auricles only, and finally by recovery. The duration of this depression varied but was shorter than after NaCl. It was of course during the phase of depression that fibrillation sometimes occurred. In sixteen hearts the behaviour of the beat was similar except that the momentary stimulation was not seen, but it may have occurred and been missed in some or all of these hearts. In twenty-one hearts neither stimulation nor depression occurred, there being a simple return to normal beating; five of these were already stationary when transferred to *PC* so that further depression was naturally impossible. This direct recovery tended to occur in those hearts which had been for shorter times in $NaCl+KCl$.

C—$NaCl+KCl \rightarrow$ PARALYSING SOLUTION 1

Material— 6 entire hearts, plasma clots.
17 entire hearts, pure salines.

The two most interesting facts in this experiment are that whereas in hearts previously treated in NaCl the second solution instantly stopped the beat and then induced transient fibrillation, after $NaCl+KCl$ the beat was frequently not instantly stopped and there was no fibrillation. Twenty-one hearts were still beating after being in $NaCl+KCl$ for periods ranging from 20 min. to over 3 hr.; in eight or ten of these the paralysing solution stopped the beat instantly or nearly so, but in the remainder beating continued for periods ranging from ½ to 3½ min. Thus in this respect the behaviour of the hearts was intermediate between that of hearts previously treated with *PC* (i.e. normal hearts) and that of hearts previously treated with NaCl. The transient return of beating frequently seen after NaCl was not observed after $NaCl+KCl$; it is possible that it may have been missed, but this is unlikely.

PART VI—THE EFFECT OF A CALCIUM-CONTAINING SOLUTION UPON HEARTS PREVIOUSLY TREATED FIRST IN NaCl AND THEN IN $NaCl+KCl$

The experiment here described shows that the ability to resist the fibrillating action of $NaCl+CaCl_2$, which is lost in NaCl, is restored by a bath in $NaCl+KCl$.

$$\begin{tikzcd} & NaCl+CaCl_2 & & \\ NaCl \arrow[ur] \arrow[dr] & & & \\ & NaCl+KCl \arrow[r] & NaCl+CaCl_2 & \end{tikzcd}$$

8 entire hearts
13 entire hearts
pure salines

The eight hearts transferred direct from NaCl to $NaCl+CaCl_2$ were controls intended to show how long hearts must be left in NaCl to ensure their being in the condition in which $NaCl+CaCl_2$ would reduce them rapidly to fibrillation. Table III shows that six clearly revealed fibrillation within 2 min., while in two fibrillation appeared after 6 and 12 min. respectively. The other thirteen hearts were transferred from NaCl to

$NaCl + KCl$ after times shown by the controls to be sufficient for them to be rapidly thrown into fibrillation if they had been transferred to $NaCl + CaCl_2$. In $NaCl + KCl$ the hearts showed the depression usual in this solution, and after varying times were transferred to $NaCl + CaCl_2$. Such beating as continued in $NaCl + KCl$ was in $NaCl + CaCl_2$ either strengthened or stopped and replaced by stronger beats, which sooner or later became beats of the whole heart. The onset of fibrillation was, in marked contrast to the control hearts, delayed for long periods in all hearts except one. This delay was so great as to make it fair to say that, in respect of the production of fibrillation by $NaCl + CaCl_2$, the hearts had been restored by the intermediate bath of $NaCl + KCl$ to practically the condition of fresh hearts.

TABLE III

$NaCl \rightarrow NaCl + CaCl_2$	
Time in NaCl	Time to fibrillation in $NaCl + CaCl_2$
43 min.	About 1½ min.
44 min.	Less than 12 min.
1 hr. 10 min.	About 6 min.
1 hr. 14 min.	At once
1 hr. 15 min.	At once
2 hr. 26 min.	No fibrillation
2 hr. 30 min.	At once
2 hr. 45 min.	1½ min.

$NaCl \rightarrow NaCl + KCl \rightarrow NaCl + CaCl_2$		
Time in NaCl	Time in $NaCl + KCl$	Time to fibrillation in $NaCl + CaCl_2$
54 min.	About 1 hr. 5 min.	At once
1 hr. 19 min.	40 min.	Over 46 min.
1 hr. 19 min.	49 min.	Well over 40 min.
1 hr. 19 min.	49 min.	Well over 40 min.
1 hr. 19 min.	1 hr. 3 min.	23 min.
1 hr. 19 min.	1 hr. 3 min.	1 hr. 38 min.
1 hr. 19 min.	1 hr. 27 min.	Over 1 hr. 40 min.
1 hr. 19 min.	1 hr. 27 min.	Over 1 hr. 40 min.
1 hr. 19 min.	1 hr. 36 min.	About 30 min.
1 hr. 19 min.	1 hr. 36 min.	Over 30 min.
1 hr. 25 min.	About 1 hr. 10 min.	About 1 hr. 30 min.
1 hr. 49 min.	About 1 hr. 25 min.	Over 1 hr. 40 min.
2 hr. 16 min.	About 1 hr. 18 min.	About 1 hr. 10 min.

PART VII—THE EFFECT OF VARIOUS SOLUTIONS UPON HEARTS PREVIOUSLY TREATED WITH $NaCl + CaCl_2$

The experiments described in this part reveal certain facts relating to the beat, especially that hearts transferred from $NaCl + CaCl_2$ to solutions containing potassium show immediate stoppage of the beat. Since the second solution in no case contained more calcium than $NaCl + CaCl_2$, such fibrillation as appeared in it must have been either calcium fibrillation surviving from $NaCl + CaCl_2$ or else must have been produced by potassium; its discussion is therefore postponed to the next paper.

A—$NaCl + CaCl_2 \rightarrow PC$

Material—16 entire hearts, pure salines.

Hearts which had been treated with $NaCl + CaCl_2$ for periods ranging from 45 min. to 3 hr. were transferred to *PC*. All were still beating at the time of the transference, and in all but one the beat was immediately stopped in *PC*; in the single exception the beat became confined to the auricles. The period of depression varied from a mere momentary stoppage to between 15 and 30 min., and was longer, the longer the treatment with $NaCl + CaCl_2$. If fibrillation was present in $NaCl + CaCl_2$ it died away in *PC*, often after a transient increase in activity; this increase is a subject for the next paper.

B—$NaCl + CaCl_2 \rightarrow NaCl + KCl$

Material— 7 auricular fragments, plasma clots.
6 entire hearts, plasma clots.
26 entire hearts, pure salines.

Taking all the material together, in twenty-eight cases beating was still going on at the time of removal from $NaCl + CaCl_2$, and in at least twenty of these the beat stopped instantly or nearly so on $NaCl + KCl$; in three it was reduced to a beat of the auricles only. Beating later returned in at least ten, and would probably have returned in more but that the hearts were, for reasons unconnected with this experiment, transferred to other solutions 10 min. after their entry into $NaCl + KCl$. In those cases in which beating had already stopped in $NaCl + CaCl_2$ the return of beating in $NaCl + KCl$ was not observed; but it might have occurred if the hearts had been left in $NaCl + KCl$ for more than 10 min.

When hearts were fibrillating in $NaCl + CaCl_2$, transference to $NaCl + KCl$ was followed by weakening of the fibrillation, which usually disappeared in 5 or 6 min. or less.

C—$NaCl + CaCl_2 \rightarrow NaCl$

Material— 6 auricular fragments, plasma clots.
51 entire hearts, pure salines (in some, with glucose in both solutions).

Thirty of the fifty-one entire hearts had ceased beating when removed from $NaCl + CaCl_2$; fifteen of these began beating again in NaCl, though in many the beat was weak and confined to the auricles, and in seven it stopped again inside a few minutes. Five of the six auricular fragments had similarly stopped beating, and the transference to NaCl restored beating to them all. Of the remaining twenty-one entire hearts, which still retained some sort of beat when removed from NaCl + $CaCl_2$, the beat was in NaCl strengthened or accelerated in eight, was unchanged in four, and was weakened or stopped in nine. Among these nine, in three the beat returned after being stopped.

When fibrillation was present in NaCl + $CaCl_2$ it gradually died away in NaCl and had usually nearly disappeared after 10 min.

D—NaCl + $CaCl_2$ → PARALYSING SOLUTIONS 1 OR 2

Material—14 entire hearts, plasma clots.

The hearts were kept in NaCl + $CaCl_2$ for times ranging from 1¾ to 5 hr. Eight still retained some sort of beat at the time of transference; with one exception transference to the paralysing solution stopped the beat at once or after a few seconds. Any fibrillation present in NaCl + $CaCl_2$ died away in the second solution, often after a transient increase in activity which will be discussed in another paper.

The most interesting event was, in five hearts, a transient return of the beat, beginning at 6, 7½, 8½, 9, 13 min. respectively after transference to the paralysing solution, and lasting in two hearts for 6 and 9 min. respectively but in the others for much shorter times, ranging from one extremely weak beat to several rather stronger beats. To these five hearts must be added the exception mentioned above; when transferred to the paralysing solution this heart did not stop beating, but the beat at once became weaker, then after 12 min. became much stronger than it had been in NaCl + $CaCl_2$, and finally at 15 min. stopped.

All the hearts finally reached a stable condition, with no beating but with fibrillation in the conus. This is the final condition of normal hearts in the same solution.

PART VIII—ANTAGONISMS BETWEEN IONS

In an earlier paper (1935 *a*) experiments were described from which it was concluded that (1) sodium antagonizes the production of fibrillation by potassium and by calcium, (2) potassium does not antagonize the production of fibrillation by calcium when sufficient potassium is present to paralyse the heart or when sufficient is present itself to produce fibrillation. The sodium-potassium antagonism was demonstrated beyond doubt, but that between sodium and calcium, and the absence of antagonism between potassium and calcium, with less certainty. The object of the present experiments was to remedy these deficiencies.

A—SODIUM-CALCIUM

The evidence (1935 *a*) that sodium antagonized the production of fibrillation by calcium was the fact that in the presence of the same concentration of $CaCl_2$ there was more beating and less fibrillation when the concentration of NaCl was high than when it was low. But the visibility of fibrillation depends on the beating, and a heart which beats without showing fibrillation may, if the beat stops or becomes slower, reveal fibrillation. It was therefore possible that, in the higher concentrations of NaCl, latent fibrillation existed, suppressed by the beating, and that what the experiment really demonstrated was an antagonism by sodium of the suppression of beating by calcium and not of the production of fibrillation by calcium.

Exp. 1

Solutions ...	NaCl	KCl	$CaCl_2$	Glucose	Material
Solution 1	0·5 %	0·06 %	0·6 %	1·85 %	6 entire hearts
Solution 2	0·8 %	0·06 %	0·6 %	—	6 entire hearts

By an hour after the hearts had been put into the solutions, beating was in both groups nearly confined to the auricles. Beating in the auricles cannot interfere with fibrillation in the ventricle, and there was definitely more fibrillation in the ventricles, and in other parts of the hearts, in solution 1 than in solution 2.

Thus, taking both old and new results into consideration, there is no doubt that calcium induces fibrillation more readily if the content of NaCl is low than if it is high.

B—POTASSIUM-CALCIUM

1—"*Consecutive*" *antagonism*

If fibrillation depended upon an excess of either potassium or calcium at the cell surface over the other, transference from a fibrillating solution having an excess of one of these ions to a fibrillating solution having an excess of the other, should be followed by a decrease in the quantity of one ion at the cell surface and increase of the other. There should therefore come a time when the ratio between the two ions should be normal and fibrillation should cease, later beginning again as the ratio again becomes abnormal.

The first step was to find a solution in which the concentration of $NaCl$ was reduced so that when high concentrations of KCl or $CaCl_2$ were added the osmotic pressure would still be below the toxic level. At the same time the level of $NaCl$ must not be so low that this in itself would prevent beating; the criterion accepted was that the solution, with its low $NaCl$, with the other salts reduced in the same proportion, and with the osmotic pressure raised by glucose to equalise with $NaCl$ 0·75 %, must maintain beating without fibrillation for at least half an hour. This was long enough because it was two or three times the duration of the experiments. The buffer was $NaHCO_3$ because this did not precipitate calcium when the $CaCl_2$ was high, as the phosphate buffer does. The solution which filled these requirements was: $NaCl$ 0·39 %, KCl 0·034 %, $CaCl_2$ 0·017 %, $NaHCO_3$ 0·03 %.

Exp. 2

Transference	$NaCl$	KCl	$CaCl_2$		$NaCl$	KCl	$CaCl_2$
(1)	0·39 %	0·7 %	0·017 %	→	0·39 %	0·034 %	0·675 %
(2)	0·39 %	0·7 %	0·017 %	←	0·39 %	0·034 %	0·675 %
(3)	0·39 %	0·8 %	0·017 %	→	0·39 %	0·034 %	0·774 %
(4)	0·39 %	0·8 %	0·017 %	←	0·39 %	0·034 %	0·774 %

Material—For (1): 5 entire hearts, pure saline.
(2): 7 entire hearts, pure saline.
(3): 2 entire hearts, pure saline.
(4): 5 entire hearts, pure saline.

All the above solutions induce fibrillation, and, in each line, the two solutions are isotonic. Hearts were placed in one solution, left there for 3 or 4 min., by which time fibrillation was active, and were then transferred to the other solution, following the arrow. In the second solution they were watched to see if there was any slackening or stoppage of the fibrillation.

The results were: (*a*) when the transference was from a solution rich in potassium to one rich in calcium, the fibrillation became, if anything, more active; there was no slackening or stoppage; (*b*) when the transference was from a solution rich in calcium to one rich in potassium there frequently was, during the first minute, a temporary lull in the activity of fibrillation, and in two cases transient but more or less complete stoppage.

2—"*Simultaneous*" *antagonism*

In these experiments the ability of potassium and calcium to antagonize one another was tested when both were present in high concentrations. A large number of experiments was performed, but, as the results were negative and confirmed the results obtained in less complete experiments described elsewhere, they will not be described in detail. The results were: (*a*) the fibrillation induced by large concentrations of potassium is not prevented or reduced in activity by any concentrations of calcium; (*b*) some evidence was found that fibrillation by large concentrations of calcium may be somewhat reduced in activity, but not prevented, by large quantities of potassium.

PART IX—DISCUSSION

A—FIBRILLATION

The experiments described show a close relation between potassium and the induction of fibrillation by calcium. Small quantities of calcium ($CaCl_2$ 0·03 %) produce fibrillation only if potassium is absent or in hearts which have been treated with potassium-free solutions (Parts III and IV); if fibrillation has been produced it is stopped again if the hearts come into the presence of potassium (Part VII A, B, D); the fibrillation produced in calcium-sensitive hearts is transient if potassium is present as well as calcium, but is not transient if potassium is absent (Part IV A and B); calcium-sensitive hearts can be made calcium-resistant by treatment with solutions containing potassium (Part VI).

Thus the ability to resist the fibrillating action of small quantities of calcium depends on potassium; in the absence of potassium the resistance is lost, in its presence it is retained or recovered.

The constant dependency of the behaviour of the hearts in calcium-containing solutions on the availability of potassium in that solution, or in solutions with which the hearts have previously been treated, clearly suggests that the loss and recovery of calcium resistance may be due to an actual loss and recovery of potassium by the cells. An accompanying paper (Watchorn and Murray) shows that if hearts are allowed to lie in potassium-free solutions they do suffer an actual loss of potassium; this being so, there is no difficulty in supposing that potassium-poor hearts will take up potassium again if they are given the opportunity. Fenn (1936 and literature there cited) and Mond and Amson (1928) supply independent evidence that potassium can be thus lost and recovered.

Potassium presents the paradox that (*a*) present in large quantities it induces fibrillation itself, (*b*) present in small quantities it opposes the production of fibrillation by calcium. This is not really very surprising, for potassium is known to have different effects according to its situation; the concentration of potassium in a muscle cell would probably paralyse the cell if it were in the external medium. It is therefore probable that the present paradox can be resolved in the same manner, and in a later paper it will be shown that, in respect of the production of fibrillation, potassium antagonises itself, and this can only mean different actions at different sites.

A number of facts lead to the conclusion that when calcium induces fibrillation it does so by some action at the surfaces of the cells, while potassium, in stopping or preventing fibrillation, acts internally. When potassium-poor (= calcium-sensitive) hearts are transferred to solutions containing both calcium and potassium the calcium sets up fibrillation more or less instantaneously while potassium stops the fibrillation only several minutes later. If it be assumed that both ions act at the same place, they must act either at the surface or internally. Either assumption meets with difficulties. (1) If both act at the surface the delay in the action of potassium as anti-fibrillator can be explained by supposing that potassium arrives at the cell surface several minutes later than calcium, but this is refuted by experiments like NaCl → NaCl + KCl, which show, by the immediate effect of potassium on the beat, that it reaches the cells as quickly as calcium. (2) In the transference NaCl → paralysing solutions, the instant stoppage of beating without stimulation shows the immediate action of potassium predominating over that of calcium, yet the action of calcium is revealed in the fibrillation, and this is not stopped for several minutes, although the solution contains about ten times as much KCl as $CaCl_2$. If both ions produce their effects on fibrillation at the same place, whether internally or externally, one would expect the potassium to overwhelm the calcium, and prevent fibrillation, but if calcium acts at the surface and potassium only after it has penetrated the cells the occurrence of a transient fibrillation is expected. (3) If both ions act at the surface, the occurrence of fibrillation depending on a predominance of superficial calcium over superficial potassium, fibrillation would not be expected when $CaCl_2$ and KCl are present in the medium in the same ratio as in *PC*, however great their absolute concentrations. But in the experiments of Part VIII fibrillation occurred when the absolute concentrations of the two salts were high even though the ratio was as in the balanced saline *PC*. (4) Fibrillation occasionally occurs in *PC* after treatment with NaCl + KCl (Part V B). Since *PC* induces fibrillation only in potassium-poor hearts, potassium must be lost in NaCl + KCl; this agrees with the general resemblance in the behaviour of the hearts to that of hearts in *PC* after treatment with NaCl for short times. Such a loss is not unexpected, for the absence of calcium would increase the permeability of the membrane, and the content of potassium within cells is higher than in NaCl + KCl. If potassium is lost from the surface in this solution it can only mean that in *PC* the presence of calcium prevents this loss. But if this is so potassium should not be lost from the surface in NaCl + $CaCl_2$; therefore, if it is potassium at the surface which opposes fibrillation by calcium, there should be no fibrillation in NaCl + $CaCl_2$. But fibrillation always occurs in this solution. Therefore it is not potassium at the surface which opposes fibrillation by calcium. If both ions be supposed to act internally, fibrillation should never occur in this experiment (NaCl + KCl → *PC*), because there is no reason why internal calcium should predominate over internal potassium; the opposite is rather to be expected, for the presence of potassium in the first solution should ensure the loss of less potassium than of calcium. (5) If potassium at the surface opposes fibrillation, there should be no fibrillation in *PC*, or in the paralysing solutions, after treatment with NaCl, because the ratio of calcium at the surface to potassium at the surface could never become abnormally high.

It is therefore concluded that fibrillation by calcium in the medium occurs when the ratio between calcium at the cell surfaces and potassium in the cell interiors is abnormally high.

This conclusion clearly accords with the results of the antagonism experiments (Part VIII), indicating that large quantities of potassium in the medium may somewhat reduce the fibrillation set up by large quantities of calcium; for the intracellular potassium may be expected to rise when the concentration of potassium in the medium is heavy, in agreement with the works of Mond and Amson and of Fenn, and this will tend to reduce the fibrillation set up by the calcium.

The occurrence of fibrillation in NaCl, in the absence of both calcium and potassium, was puzzling, for sodium antagonizes the production of fibrillation by potassium and calcium (1935 *a* and Part VIII). Fibrillation in NaCl resembled that in NaCl + $CaCl_2$ very closely, differing only in being much less active and, in young hearts, in occurring much less constantly. In both NaCl and NaCl + $CaCl_2$ the beat gradually died away and fibrillation appeared before and continued after the cessation of beating. During fibrillation in either solution the hearts became increasingly opaque; this was never seen in NaCl + KCl. Upon transference of hearts fibrillating in NaCl + $CaCl_2$ to NaCl the fibrillation gradually weakened, but might not completely stop, and on transference from NaCl to NaCl + $CaCl_2$ the fibrillation was greatly increased. Transference of fibrillating hearts from either NaCl + $CaCl_2$ or NaCl to NaCl + KCl stopped the fibrillation. All these facts suggest that the fibrillation in NaCl was caused by the same conditions as in $NaCl + CaCl_2$, viz. a high ratio superficial calcium/internal potassium, and was more active in $NaCl + CaCl_2$ because the calcium in the medium kept the superficial calcium higher than in NaCl.

B—BEATING*

1—*Influence of a change from less to more calcium* (5, 9, 10, 16, 17)

The beat is stimulated in all cases and whether the increase is absolute or relative, i.e. same amount of calcium but less potassium, except when the calcium in the second solution is overwhelmed by much larger quantities of potassium (these cases are transferences from calcium-free media to paralysing solutions containing calcium, 11, 12, 18).

Subsequent behaviour depends on the treatment to which the hearts have previously been subjected.

a—Potassium-rich hearts.

In potassium-rich hearts, i.e. hearts which have an adequate supply of potassium because of the presence of KCl in the first solution, the improved beating is maintained for long periods (*PC* → $NaCl + CaCl_2$, NaCl + KCl → $NaCl + CaCl_2$, NaCl → $NaCl + CaCl_2$ after short times in NaCl). The experiment NaCl + KCl → *PC* (17), in which the beat is usually stopped after stimulation, is only an apparent exception, and supports the view already advanced in connection with the occurrence of fibrillation in this transference, that potassium is lost in NaCl + KCl, so that the hearts are to some extent potassium-poor.

b—Potassium-poor hearts.

When the hearts are potassium-poor, having been previously treated with solutions lacking potassium, or with NaCl + KCl, the beat is always stopped or depressed after initial stimulation (9, 10, 17). If the second solution contains no potassium the stoppage is permanent (NaCl → $NaCl + CaCl_2$) but if potassium is supplied in the second solution the beat is stopped after stimulation but later returns and is then maintained for long periods (NaCl → *PC*, NaCl + KCl → *PC*).

CONCLUSIONS—Calcium in the medium favours beating, but its beneficent effect depends on the availability of potassium. If the heart already contains sufficient potassium, the improved beat is maintained; if not, the beat is stopped, but is recovered again as potassium is taken up from the solution. Its effect being instantaneous, calcium acts at the surface. Potassium acts internally because: (*a*) Its action in enabling the beat to return after stoppage (in potassium-poor hearts) occurs after fibrillation has died away and some minutes at least after the beat has stopped, i.e. when internal potassium has been taken up again. (*b*) If it acts at the surface there is no reason why beating should stop when the second solution contained potassium, because potassium is immediately available at the surface, and is known, from the instant action of potassium in some experiments, to reach the surface as quickly as calcium. The occurrence of stimulation therefore shows that a rise in the ratio superficial calcium/internal potassium favours beating, but the immediately following stoppage in potassium-poor hearts shows that too great a rise stops beating.

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* Figures in brackets refer to the numbers of experiments in the tabular summary, Table IV.

2—*Influence of a change from less to more potassium* (4, 6–8, 11–15, 18, 20, 21, 23)

The beat is always stopped or depressed without previous stimulation. This occurs whether the change involves an absolute increase in the amount of potassium, or a relative increase (i.e. same amount of potassium but less calcium).

a—Potassium-rich hearts (4, 6–8, 18).

The stoppage or depression is gradual, the process occupying several minutes.

i—One experiment, *PC* → NaCl + KCl (4). This experiment was especially interesting. After stoppage, beating returns and is maintained for long periods; thus, the stoppage must be caused by a change which is later reversed. Transference to the calcium-free medium involves an increase in superficial potassium and a decrease in superficial calcium, but the change in the ratio between these will not be later reversed. Several considerations have already suggested that internal potassium is lost in NaCl + KCl. If so, the ratio superficial calcium/internal potassium, at first lowered by displacement of calcium, will rise again by loss of internal potassium; no other ratio will thus reverse the direction of its change. It is known that a rise in this ratio stimulates beating; therefore a fall will depress, and a subsequent rise to somewhere near the normal level will restore the weak beating which is actually seen.

ii—These experiments (*PC* → paralysing solutions 1 and 3, with and without calcium, and NaCl + KCl → paralysing solution 1) differ from that just discussed chiefly in the larger quantities of potassium in the second solutions. Stoppage is gradual and the beat does not return, either because the larger quantities of potassium prevent the loss of internal potassium, or displace too much superficial calcium, or because the ratio of superficial to internal potassium is too high.

b—Potassium-poor hearts (11–15, 20, 21, 23, NaCl → paralysing solutions 1 and 3, with and without calcium, and → $NaCl + KCl$; $NaCl + CaCl_2$ → paralysing solutions 1 and 2, and → *PC*, and → $NaCl + KCl$).

The stoppage is sudden and rapid. Since potassium-rich hearts stop slowly and potassium-poor hearts rapidly, in solutions whose content of potassium is responsible for the stoppage, potassium in the cells opposes the action of potassium in the medium. Since potassium often stops the beat instantly, its action as depressor must be at the surface, opposed by potassium within the cells. Thus stoppage is caused by a rise of the ratio superficial potassium/internal potassium. It is important to note that stoppage is not caused by a rise in the ratio superficial potassium/superficial calcium because beating stops more rapidly in the transferences $NaCl + CaCl_2$ → $NaCl + KCl$ (21), or → a paralysing solution (23), than in the transferences *PC* → $NaCl + KCl$ (4) or → a paralysing solution (6–8), although superficial calcium must be as high, and probably higher, after $NaCl + CaCl_2$ than after *PC*.

i—*Calcium available in first, second, or both solutions* (11, 12, 20, 21, 23). In all these experiments there was a return of beating after stoppage.

α—In one group (20, 21) the beat returns and is maintained for long periods. Here the experiment $NaCl + CaCl_2$ → *PC* (20) is interesting because it affords proof that the instantaneous stoppage or depression of the beat in *PC* is due to a rise in superficial potassium/internal potassium. It is known (Watchorn and Murray) that potassium is lost in $NaCl + CaCl_2$, and since the only difference between the first and second solutions is the presence of potassium in the second, the stoppage must represent the effect of potassium on a potassium-poor heart. Since the rapidity of the stoppage is the consequence of the poverty of the hearts in potassium, and since the change responsible for the stoppage is later reversed as the hearts take up potassium and beat again, the effective ratio must be superficial potassium/internal potassium. The stoppage cannot be caused by a rise of superficial potassium/superficial calcium because, in *PC*, it would be impossible for this ratio to become abnormally high, especially after $NaCl + CaCl_2$.

Similar considerations apply to the transference $NaCl + CaCl_2$ → $NaCl + KCl$.

β—(11, 12, 23; NaCl → paralysing solutions 1 and 3, NaCl + $CaCl_2$ → paralysing solutions 1 and 2). The events here are: instant stoppage of beating; onset and cessation of fibrillation; return and final cessation of beating. The first stoppage is clearly to be attributed to the rise of superficial potassium over internal potassium; rise of superficial calcium probably helps, but is not the sole factor because in the experiment NaCl + $CaCl_2$ → paralysing solution 1 (23) *it* decreases but the beat stops as in the other experiments. The fibrillation is of course due to the rise of superficial calcium/internal potassium, but the rise of superficial potassium probably helps; another paper will show that potassium fibrillation is induced by a rise in the ratio superficial potassium/internal potassium. The transient return of beating can only be attributed to the rise of internal potassium, lowering, for a time, the ratio of superficial to internal potassium to within the limits of beating. The final stoppage may be due to either (*a*) displacement of superficial calcium by potassium so that the ratio of these two to the internal potassium varies beyond the limits of beating, or (*b*) further attachment of superficial potassium so that its ratio to internal potassium rises above the limits of beating.

ii—*Calcium not available in either solution* (13, 14, 15; NaCl → NaCl + KCl; NaCl → paralysing solutions 1 and 3, without calcium). The beat, after rapid stoppage, does not return. The stoppage is to be attributed to the same factor as the first stoppage in i*β*, i.e. to the rise of superficial potassium/internal potassium. There is no return of beating, obviously because there is no calcium, causing superficial calcium to be very low relative to internal potassium. One point of some importance is that the stoppage is less rapid in the transference from NaCl to paralysing solution 3 without calcium (15) than in the transference from NaCl to the same solution with calcium (12). It follows that the first stoppage in paralysing solution 3 after NaCl (and by implication in the other experiments of the group i*β*) is accelerated by a high superficial calcium/internal potassium, and therefore is not caused by this ratio changing in the opposite direction.

The conclusions in regard to beating are given in the Summary, paragraph 9.

PART X—SUMMARY

The principal experimental results are summarized in Table IV. The numbers [in square brackets] in the summary refer to the numbers of experiments in this table.

1—The effect of various solutions on the normal heart was studied [1–8]; in the majority of experiments [9–23] two or three experimental solutions were used consecutively with the object of examining the effect of each solution on hearts previously treated with various other solutions.

2—Hearts which had been treated with media lacking potassium were readily thrown into fibrillation by small quantities of calcium ($CaCl_2$ 0·03 %) [9–12], but if small quantities of potassium (KCl 0·06 %) were present in the first solution, this protected them against the fibrillating action of small quantities of calcium in the second [16–18].

3—When hearts treated with solutions lacking potassium were transferred to solutions containing calcium, but not potassium, fibrillation occurred and was not transient [9]. But if the second solution contained both potassium and calcium, fibrillation was transient, ceasing in a few minutes [10–12].

4—When hearts treated with a solution lacking potassium were first transferred to a solution containing potassium and thence to a solution containing calcium but lacking potassium, it was found that the potassium in the second bath enabled the hearts to resist the fibrillating action of the calcium in the third bath [19].

5—The observations recorded in an accompanying paper (Watchorn and Murray) show that potassium was lost from hearts when they were treated with solutions lacking potassium. There is indirect evidence that potassium was also lost in the presence of KCl 0·06 % when calcium was absent (the solution NaCl + KCl) [17].

6—It is concluded (Part IX A) that fibrillation induced by calcium in the medium depends on an abnormally high ratio between calcium at the cell surfaces and potassium in the cell interiors.

7—Stimulation of the beat (strengthening, or restoration after loss) occurred when the hearts were transferred to a solution containing more, or relatively more, calcium than was present in the first solution [5, 9, 10, 16, 17, 19], unless the effect was prevented by the presence of large quantities of potassium [11, 12, 18]. If the hearts were not potassium-poor, the improved beating was maintained for long periods [5, 16, 17, 19], but if the hearts were potassium-poor the stimulation was at once followed by stoppage of the beat. If the second solution contained no potassium this stoppage was permanent [9]; but if the second solution contained small quantities of potassium the beat subsequently returned [10].

8—When hearts were transferred to solutions containing more, or relatively more, potassium than the first solution (but not more calcium unless potassium greatly preponderated over it), the beat was depressed or stopped without stimulation [4, 11–15, 18, 20, 21, 23]. The inhibitory effect of potassium appeared much more rapidly if the hearts were potassium-poor than if they were not.

9—From these and a large number of other facts it is concluded (Part IX B) that the influence on beating of calcium and potassium in the medium depends on the balance between the two ratios: calcium at the cell surface/potassium in the cell interior, and potassium at the cell surface/potassium in the cell interior, but that the ratio between calcium at the cell surface and potassium at the cell surface is without influence, except indirectly by altering the balance of the two effective ratios. Relative increase of superficial calcium over internal potassium stimulates beating; but if the increase is too great it stops beating and fibrillation is induced. Decrease of this ratio depresses or stops beating. Increase of superficial potassium relative to internal potassium depresses beating.

TABLE IV—SUMMARY OF PRINCIPAL EXPERIMENTAL RESULTS

For meanings of symbols see Table I and Part I of text.

Experiment	Part	Results
1. *PC* → NaCl	II	Gradual dying away of beat, gradual onset of weak fibrillation
2. *PC* → KCl	II	Rapid stoppage of beat, active fibrillation
3. *PC* → $CaCl_2$	II	Do.
4. *PC* → NaCl + KCl	III	Beat dies away in a few minutes—later weak beat returns and is maintained indefinitely
5. *PC* → NaCl + $CaCl_2$	III	Gradual dying away of beat, gradual onset of fibrillation—death
6. *PC* → paralysing solution 1	IV B	Gradual dying away of beat, thereafter stationary except fibrillation in conus
7. *PC* → paralysing solution 3	IV B	Do.
8. *PC* → paralysing solution 3 without calcium	IV C	Gradual dying away of beat, thereafter stationary
9. NaCl → NaCl + $CaCl_2$	IV A	Stimulation of beat—stoppage of beat—non-transient fibrillation—death
10. NaCl → *PC*	IV B	Stimulation of beat—stoppage of beat—transient fibrillation—return of beat

TABLE IV—(*continued*)

Experiment	Part	Results
11. NaCl → paralysing solution 1	IV B	Stoppage of beat—transient fibrillation—transient return of beat—stoppage of beat, no further return of beat
12. NaCl → paralysing solution 3	IV B	Do.
13. NaCl → NaCl + KCl	IV C	Beat and fibrillation stopped; no induction of fibrillation; beat not seen to return
14. NaCl → paralysing solution 1 without calcium	IV C	Do.
15. NaCl → paralysing solution 3 without calcium	IV C	Beat stopped (less rapidly than when calcium present), no fibrillation; transient return of beat in only one case; permanent stoppage of beat
16. NaCl + KCl → NaCl + $CaCl_2$	V A	Strengthening or restoration of beat—prolonged maintenance of beat—ultimate fibrillation
17. NaCl + KCl → *PC*	V B	Stimulation of beat—depression or stoppage of beat—rarely fibrillation—return of beat (earlier phases often omitted)
18. NaCl + KCl → paralysing solution 1	V C	Beat stopped but not instantly; no fibrillation; no return of beat; permanent stoppage of beat
19. NaCl → NaCl + KCl → NaCl + $CaCl_2$	VI	In NaCl + $CaCl_2$: Strengthening or restoration of beat—prolonged maintenance of beat—ultimate fibrillation
20. NaCl + $CaCl_2$ → *PC*	VII A	Instant stoppage or depression of beat—transient fibrillation (probably surviving from NaCl + $CaCl_2$)—return of beat
21. NaCl + $CaCl_2$ → NaCl + KCl	VII B	Stoppage of beat and of fibrillation—return of beat
22. NaCl + $CaCl_2$ → NaCl	VII C	Restoration, strengthening, or stoppage of beat, more or less complete dying away of fibrillation
23. NaCl + $CaCl_2$ → paralysing solutions 1 or 2	VII D	Virtually instant stoppage of beat—transient fibrillation (probably surviving from NaCl + $CaCl_2$)—transient return of beat—permanent stoppage of beat
24. Antagonism experiments	VIII	In respect of fibrillation: sodium antagonizes potassium and calcium; calcium does not antagonize potassium; large quantities of potassium probably weakly oppose production of fibrillation by large quantities of calcium

REFERENCES

Fenn, W. O. 1936 *Physiol. Rev.* **16**, 450.
Fredericq, L. 1929 *Arch. int. Physiol.* **31**, 264.
—— 1931 *Arch. int. Physiol.* **34**, 110.
Mond, R. and Amson, K. 1928 *Pflug. Arch. ges. Physiol.* **220**, 69.
Murray, P. D. F. 1934 *a* *Proc. Roy. Soc.* B, **115**, 380.
—— 1934 *b* *Arch. int. Physiol.* **40**, 243.
—— 1935 *a* *Proc. Roy. Soc.* B, **116**, 434.
—— 1935 *b* *Proc. Roy. Soc.* B, **116**, 452.
Olivo, O. 1924 *Arch. Fisiol.* **22**, 3.
Watchorn, E. and Murray, P. D. F. 1938 *Proc. Roy. Soc.* B, **124**, 446.

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Loss of Potassium from Embryonic Chick Hearts in Potassium-free Media with and without Calcium

BY E. WATCHORN AND P. D. F. MURRAY

Sir William Dunn Institute of Biochemistry and Strangeways Research Laboratory, Cambridge

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Experiments already described (Murray 1938) led to the inference that the cells of the chick embryonic heart lose potassium in potassium-free media. The experiments here described provide direct evidence of this.

METHOD

The hearts were dissected out of 2½–3 day chick embryos and placed in the solution *PC* (Table I) until they had started to beat. They were then thoroughly washed, and were allowed to lie for 5 min. (2 min. in Exp. 1) in the last wash. This last wash is called control A. The solutions used for washing were from the same flasks as the experimental solution. After their passage through control A the hearts were transferred to 2 c.c. of the experimental solution in a Jena watch-glass. After various times in this the hearts were discarded and both the experimental solution and control

A were collected. If the experiment extended over more than 1 day the experimental solution and control A were used over again day by day until all the hearts in the experiment had passed through them. The use of control A was necessary for two reasons: (*a*) to show that potassium was not still being washed out of the intercellular spaces at the end of washing, (*b*) in experiments lasting over several days the washing solution was fresh each day, but the experimental solution was of course not changed. Hence any small amount of potassium being carried over from the last wash would accumulate in the experimental solution because of the daily increment and might seriously affect the result; but by leaving the hearts for several minutes in the last wash (control A) and by not changing it for fresh on successive days, any such increase would be detected in that solution. In addition to control A, a daily sample (control B) was taken from the same flasks as the solutions used for washing. Details of the solutions are given in Table I; a phosphate buffer was always used.

TABLE I—G./100 g. water

Solution	NaCl	KCl	$CaCl_2$
PC	0·75	0·06	0·03
$NaCl + CaCl_2$	0·75	—	0·03
$NaCl + KCl$	0·75	0·06	—
NaCl	0·75	—	—

As soon as a few of the hearts had stopped beating or (in the case of Exp. 3) either beating or fibrillating, they were all removed from the experimental solution and discarded, and the solutions collected. This was done because stationary hearts might be dead and if so might liberate more potassium than during life. Stoppage of the experiment when a few had become stationary eliminated this difficulty because there is an interval between cessation of visible activity and death.

Finally, the experimental solution, control A, and the samples constituting control B, were examined for the presence of potassium. This was done by means of the cobalti-nitrite reagent. Two c.c. of this were added to 1 c.c. of each solution tested, mixed, and allowed to stand for 45 min. After centrifuging at 3500 revolutions per minute for 20 min. the supernatant fluid could be safely poured off. By this means the presence of even a trace of potassium was plainly revealed by the potassium cobaltinitrite crystals remaining in the tube. When an appreciable amount of potassium was present attempts were made to estimate it by means of Jacobs and Hoffman's colorimetric method. It was never possible to make a match in the colorimeter, but an approximation could be obtained by naked-eye comparison with suitable standards.

Exp. 1—Thirty-four hearts were used and the experiment was completed in 1 day. Washing was done through four Petri dishes each containing NaCl. Control A was NaCl in a watch-glass; the hearts were left in it for 2 min. The experimental solution was 2·2 c.c. of NaCl in a watch-glass. The hearts were left in the experimental solution for 1¾ hr., after which time most displayed weak beating but a few were stationary. The fluids were then collected; unfortunately about half the experimental saline was accidentally lost. Chemical examination gave the following results:

Controls A and B: no trace of potassium.

Experimental: potassium definitely present, in amounts too small for accurate measurement, but estimated at 25–50γ/c.c.

Exp. 2—The object was to confirm the results of Exp. 1 and to see if the hearts would lose potassium in the presence of calcium.

The experiment extended over 6 days. Ninety-six hearts were washed and subsequently allowed to lie in NaCl and ninety-six were similarly treated in NaCl + $CaCl_2$. The time in control A was 5 min. On the first 2 days washing was by transference of the hearts through six watch-glasses of NaCl or NaCl + $CaCl_2$, on the remaining 4 days through eight watch-glasses. The two groups were left in the experimental salines for equal times, but the times varied on the different days, depending on the duration of activity in NaCl. In nearly all cases beating was still going on in NaCl + $CaCl_2$ at a time when a few hearts in NaCl had become stationary and the solutions were collected. Table II summarizes numerical data.

TABLE II

Day ...	1	2	3	4	5	6
No. of hearts in each group	17	9	16	17	16	21
Times in each experimental solution (mins.)	54	43	73	74	92	81

The result of the chemical examination of the solutions was:

NaCl. Control A: slight traces of potassium.

Control B: very slight traces of potassium.

Experimental: potassium definitely present, not accurately measurable, but approximately three times as much as in Controls A and B.

$NaCl + CaCl_2$. All three solutions: very slight traces of potassium, perhaps more in the experimental than in the control solutions, but the difference was very much less than in the case of NaCl alone.

Exp. 1 was thus confirmed, but no reliable evidence was found that potassium was lost in the presence of calcium. If it was lost in the presence of calcium, this happened more slowly than in the absence of calcium.

Exp. 3—In Exp. 2 it was noticed that the hearts in $NaCl + CaCl_2$ were always in better condition at the end of the experiment than those in NaCl; it was therefore possible that if the hearts were left in $NaCl + CaCl_2$ for longer times, and if more of them were used, a positive result might be obtained. The experiment was therefore repeated, over six days, with 222 hearts, all of which were used for $NaCl + CaCl_2$. Table III gives the numerical data.

TABLE III

Day ...	1	2	3	4	5	6
No. of hearts	36	37	35	41	38	35
Time in experimental solution (hr. min.)	2.23	2.15	3.0	3.30	3.0	3.30

The method was modified in one respect: the process of washing was carried through in a glass tube, the hearts remaining in the tube and the solution being changed twelve times. The thirteenth wash was control A, and hearts were left in it for 5 min. The final soaking in the experimental solution was carried out in a watch-glass as before, because it was necessary to use a microscope. The hearts were discarded and the solutions collected when a few hearts had stopped both beating and fibrillating.

The result of the chemical examination was:

Controls A and B: no trace of potassium.

Experimental: potassium present, not measurable, but estimated at 75γ/c.c.

Exp. 4—Observations described in the accompanying paper (Murray 1938) gave reason for believing that hearts lose potassium in calcium-free media even though potassium is present. In this experiment the solution used was $NaCl + KCl$, the number of hearts was fifty-five, and the object was to detect an increase in the amount of potassium in the $NaCl + KCl$ after the hearts had been soaked in it. The chemical examination seemed to show slightly more potassium after the hearts had passed through the solution than was present in it before, but the difference was very slight and lay within the limits of possible error. It is naturally more difficult to detect an increase in the potassium in a solution already containing it than to recognize the presence of potassium in a solution previously potassium-free, and to this difficulty must be added the fact that the biological observations reported in the other paper indicate a smaller loss of potassium in potassium-containing than in potassium-free solutions.

CONCLUSIONS

Exps. 1 and 2 show that hearts bathed in NaCl lose potassium. In Exp. 3 it is shown that the same happens in NaCl + $CaCl_2$; but comparison between the two groups in Exp. 2 (NaCl and NaCl + $CaCl_2$) shows that the loss of potassium is less rapid if calcium is present than if it is absent.

REFERENCES

Jacobs and Hoffman 1931 *J. Biol. Chem.* **93**, 685.
Murray, P. D. F. 1938 *Proc. Roy. Soc.* B, **124**, 421.

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The Effect of Freezing on Bacteria

By R. B. Haines

Low Temperature Research Station, Cambridge

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It has been known for many years that cultures of bacteria can be cooled to very low temperatures without destroying all the cells present (see, for example, MacFadyen and Rowland 1900). More recently the preservation of bacteria and sera by rapid freezing and drying has come into general use. The fundamental changes accompanying these processes have not so far been investigated in much detail. This paper presents the results of experiments on the effects of cooling and freezing at various temperatures on a number of bacterial species, and an attempt to correlate the observed effects of freezing on viability with the effect of freezing on the cellular proteins of *B. pyocyaneus* [*Pseudomonas aeruginosa*].

Hypotheses previously put forward to explain the observed effects of low temperatures on living cells may be briefly summarized under three headings:

1—The capillary theory. In capillary spaces water freezes below 0° C. (Zsigmondy 1911; Fisher 1924), and it has been suggested that cells surviving exposure to low temperature have not in fact been frozen (Morrison 1924).

2—The mechanical destruction theory. Cells are said to be disintegrated by mechanical compression either on freezing or thawing (Lepeschkin 1924), or the surface of the cell is said to be destroyed (Maximov 1912).

3—The "chemical" or "desiccation" theory. It is known that in some cases water is removed from the cell and freezes round it. This may lead to concentration of solutes within the cell, change in pH, and consequent denaturation of one or more of the native proteins of the cell. The work of Moran (1929), Smith (1929) and Finn (1932) has shown that there is in fact a critical temperature at $-2°$ C. for mammalian and frog muscle which can be explained on this basis.

It is not easy to devise critical experiments to decide between these alternatives in the case of bacteria, but preliminary tests suggested that positive evidence could be obtained on hypothesis 3. Attention has been, therefore, chiefly devoted to this.

THE EFFECT OF FREEZING AND THAWING

Aqueous suspensions of young cultures of bacteria (usually 20 hr. cultures and never older than 24 hr.) were used. Two series of experiments were made. In the one a single drop of the bacterial suspension was placed on a clean, sterile cover-slip, using a calibrated dropping pipette, and the drop frozen by placing the cover-slip inside a Petri dish previously cooled on solid carbon dioxide at −70° C. Under these conditions freezing was extremely rapid. In the other case five drops of the suspension were introduced into the bottom of a sterile ignition tube, taking care not to allow the suspension to trickle down the side of the tube, and the tubes frozen by plunging into the solid carbon dioxide. In either case, after freezing, the suspensions were thawed at room temperature, suitably diluted and plated on agar. (With these small volumes thawing was completed in a few minutes.) The growth of the organisms being somewhat delayed after this treatment, several days' incubation were allowed. In certain cases total counts were made, using the Wilson technique (1922) with dark-ground illumination, and the organisms were also examined with high power dark-ground illumination and in stained preparations. Several experiments were made with each organism. In general, organisms could be recovered from the slips or tubes before freezing with an accuracy such that the standard deviation of the mean was never greater than ± 10 % and was often less. After freezing, however, the results were more erratic, a standard deviation of ± 20 % being obtained for the slips and for the tubes ± 35 %. Within these limits, for any given organism the ratio of viable cells before and after freezing was constant and reproducible. In certain instances the resistance of spores was compared with that of vegetative cells of the same organism. The suspension of spores was made by emulsifying the 3-day growth on agar, and heating to 80° C. for 10 min., followed by vigorous shaking. Microscopic examination showed that few if any vegetative cells remained, and the spores were not clumped. A suspension of vegetative cells of the same organism was made by growing in nutrient broth for 24 hr., centrifuging and resuspending in water with vigorous shaking.

A summary of the results of rapid freezing and thawing is given in Table I.

Organisms thus vary in their susceptibility to freezing and thawing, *B. pyocyaneus* being one of the most sensitive, while spores in general are but little affected. Vegetative cells of *B. mesentericus*, the spores of which are comparatively resistant, are fairly susceptible. *Staphylococcus aureus* also seems to be resistant but figures with Staphylococci may be misleading, since vigorous shaking of suspensions of these bacteria fails to break up all the clumps. It is known that freezing may break up clumps in some cases, and this would lead to a spuriously high ratio. A lower mortality was obtained with freezing on slips than with freezing in tubes.

TABLE I—RATIO OF VIABLE CELLS BEFORE AND AFTER RAPID FREEZING AT −70° C. IN SOLID CARBON DIOXIDE, AND THAWING AT ROOM TEMPERATURE

Organism ...	*Bact. coli* Esch.	*Staph. aureus*	*B. pyocyaneus* [*Pseudomonas aeruginosa*]	*Achromobacter*	*Sacch. cerevissiae*	Spores *B. mesentericus*	Vegetative cells *B. mesentericus*	Spores *B. cereus*	Spores *B. megatherium*
				Frozen in tubes					
No. of experiments	4	3	12	3	3	6	—	3	—
Ratio	0·58 ± 0·13	0·96 ± 0·04	0·18 ± 0·05	0·39 ± 0·08	0·11 ± 0·05	0·73 ± 0·16	—	1·14 ± 0·08	—
				Frozen on slips					
No. of experiments	—	4	4	—	—	4	4	—	4
Ratio	—	0·95 ± 0·03	0·49 ± 0·03	—	—	0·90 ± 0·10	0·41 ± 0·17	—	0·95 ± 0·10

In the above experiments the rate of freezing was roughly constant. It is difficult to control the rate of freezing with small drops, but it is assumed that freezing at −70° C. is more rapid than freezing with seeded drops at −5 and −20° C. A comparison was then made between the ratios of organisms before and after freezing at these temperatures (Table II).

TABLE II—EFFECT OF THE RATE OF FREEZING

Ratio of viable cells (*B. pyocyaneus*) before and after freezing and thawing.

Ten experiments in each case

Frozen at	Ratio
−70° C.	0·55 ± 0·10
−20° C.	0·52 ± 0·05
−5° C.	0·48 ± 0·05

Over this range, therefore, no evidence could be obtained that the rate of freezing had any definite effect. This result is in opposition to the "mechanical destruction" theory, since the larger ice crystals obtained in slow freezing might be expected to exert a greater disruptive effect on the cell, assuming intracellular crystallization.

Two possible effects might be anticipated, according to whether formation of ice occurs (1) inside or (2) outside the cell.

(1) On purely physical grounds the cell should be larger in the frozen state. The increase in volume in this case can be shown, however, to be so slight as probably to escape detection in small cells. The following assumptions are made: (*a*) the organism contains 80 % water, (*b*) as with muscle 75 % of the water is frozen at -3° C. (Moran 1935), (*c*) density of the organism unity, (*d*) 1 g. of water in changing to ice expands by 0·09 c.c. It follows that the increase of volume of the cell would be 5·4 %. Taking the largest cell used in the present work, Saccharomyces, with a diameter of $8{\cdot}2\mu$, it can be seen that the increase would not be detectable.

(2) On the other hand, if water is withdrawn from the cell to freeze round it, the cell might shrink. In either case, it ought to be possible to demonstrate rupture of the cell, if it has occurred.

Two sets of experiments were made on this point. Examination of suspensions before and after freezing, using a $\frac{1}{12}$ in. oil-immersion lens N.A. 1·37 and very thin slides, with a dark-ground illuminator, failed to show any appreciable degree of rupture of the cells, either with *B. pyocyaneus*, vegetative cells of *B. mesentericus*, *Saccharomyces ellipsoideus* or *S. cerevissiae*. The total count, made with a Wilson chamber and dark-ground illumination, had not diminished after freezing and thawing, in the case of *B. pyocyaneus*.

Secondly, frozen films of organisms were examined directly under the microscope. A few c.c. of a broth or malt agar culture of the organism were placed in a watch-glass, and by carefully withdrawing a platinum loop of about 0·5 cm. diameter a thin film was obtained which was then frozen by holding over solid carbon dioxide. It was mounted in plasticene on the microscope stage in a room at -3° C. and examined with a $\frac{1}{8}$ in. objective. With bacteria it was difficult to obtain definite results, but the cells appeared to be lying undistorted among crystals of ice about ten times the length of the organism. The larger cells of *Saccharomyces cerevissiae* could, however, be measured with a Ramsden micrometer, fifty fully grown cells being selected at random, omitting obvious buds, and the greatest diameter and another perpendicular to it, measured. Unfrozen cells, frozen cells, and frozen and thawed cells from the same culture were measured. An example of the type of result obtained is given in Table III.

No significant difference could therefore be detected in the overall size of the cells before and after freezing, or in a frozen film. If water is removed from the cell on freezing, the membrane must be sufficiently rigid to maintain the shape of the cell unaltered. This would be in agreement with the findings of Wámoscher (1930) and Knaysi (1930), the former of whom found it difficult to distort bacterial cells with micro-dissecting needles, while the latter gave diagrams indicating that even when the cytoplasm was drawn in after immersing the cells in 25 % sodium chloride, the membrane retained its original position (*B. subtilis*).

TABLE III—EFFECT OF RAPID FREEZING OF FILMS OF *SACCHAROMYCES CEREVISSIAE* ON THE SIZE OF THE CELL

Mean of 50 measurements. In scale divisions, 1 division = 0·3 μ

Before freezing		In frozen state		After freezing and thawing	
Long diameter	Short diameter	Long diameter	Short diameter	Long diameter	Short diameter
27·2	19·3	28·3	20·1	27·7	18·5

Frozen and unfrozen cells of *B. pyocyaneus* have been examined by a variety of staining methods, but no definite evidence of any cytological change has been obtained.

It seems, therefore, that the balance of evidence obtained by the above experiments is against hypothesis (2), but will not differentiate between (1) and (3). Further evidence was sought by turning to the effects of prolonged storage in the frozen state.

THE EFFECT OF STORING FROZEN SUSPENSIONS AT TEMPERATURES FROM −2 TO −20° C.

Small glass tubes, containing 0·1 c.c. of bacterial suspension, filled and frozen rapidly at −70° C. as previously described, were well corked to prevent evaporation, and stored in the frozen state at temperatures of −1, −2, −5, −10 and −20° C. for prolonged periods. From time to time several of the tubes were withdrawn, and the number of viable organisms surviving determined by plating. In general, each count at each temperature was made in triplicate, since considerable variation occurred from tube to tube. Experiments were made with *B. pyocyaneus*, *Bact. coli*, *B. mesentericus* (spores) and *Staphylococcus aureus*. The results are summarized in figs. 1–4. Briefly, it was found that at −20° C. organisms died out slowly, about 25 % of the original population of *Bact. coli* for example, being alive after 163 days at −20° C. At −1 and −2° C., on the other hand, death occurred comparatively rapidly, the viable population with this organism at −2° C. being reduced to 4 % in 11 days. The effect was most marked with *B. pyocyaneus*, the organism that showed the greatest mortality on freezing, much less with *Staphylococcus aureus*, while spores of *B. mesentericus* suffered no change at any temperature, 50–100 % being viable after 133 days at −20 or −2° C.

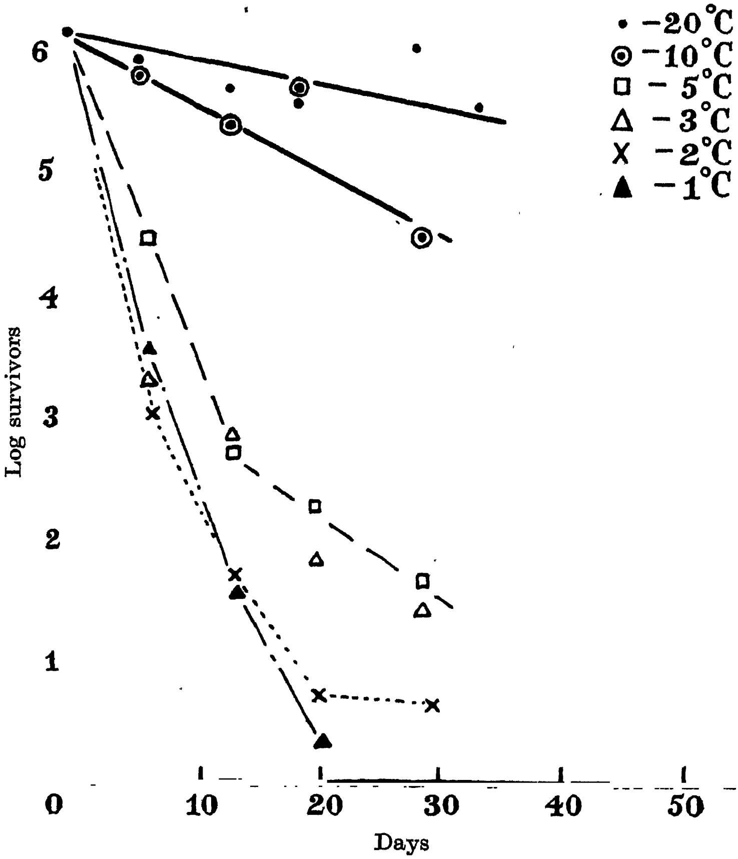

Fig. 1—Death of *B. pyocyaneus* in frozen suspensions stored at different temperatures.

These findings suggest that there is a critical temperature of storage in the frozen state for bacteria, analogous with muscle, near −2° C. To test this point further, tubes of suspension frozen and kept at −20° C. and showing the slow rate of death characteristic of that temperature, were transferred to −2° C. The following is a typical result (Table IV).

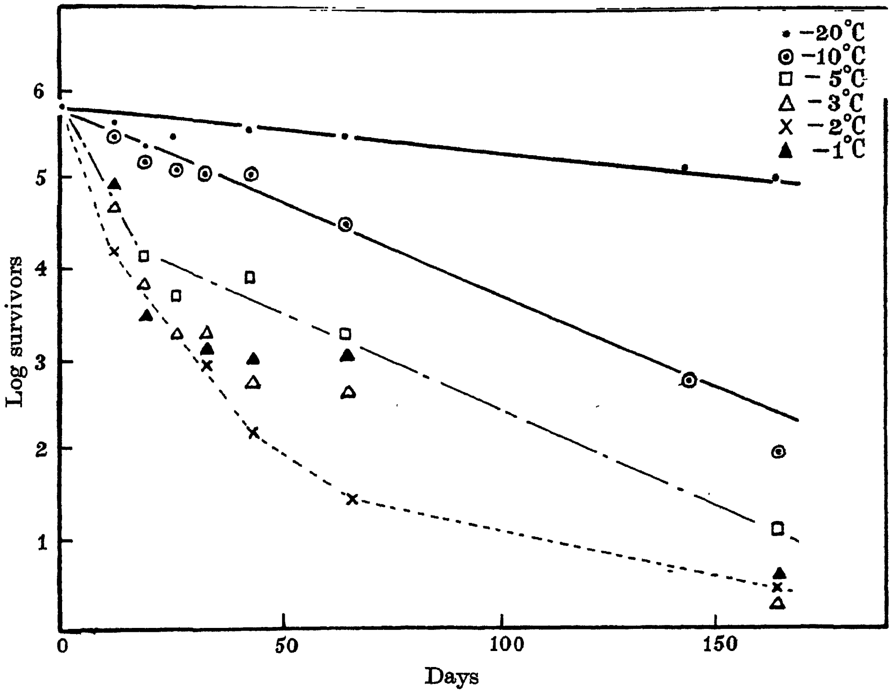

FIG. 2—Death of *Bact. coli* in frozen suspensions.

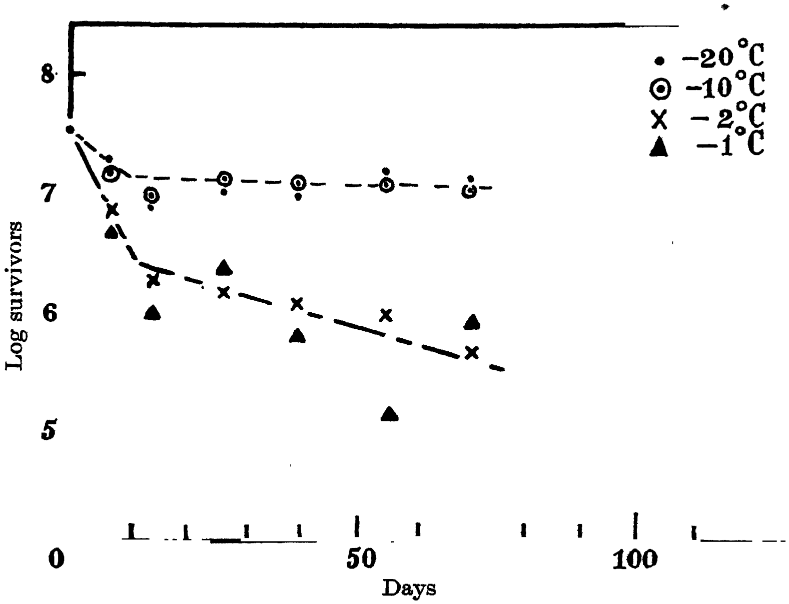

FIG. 3—Death of *S. aureus* in frozen suspensions.

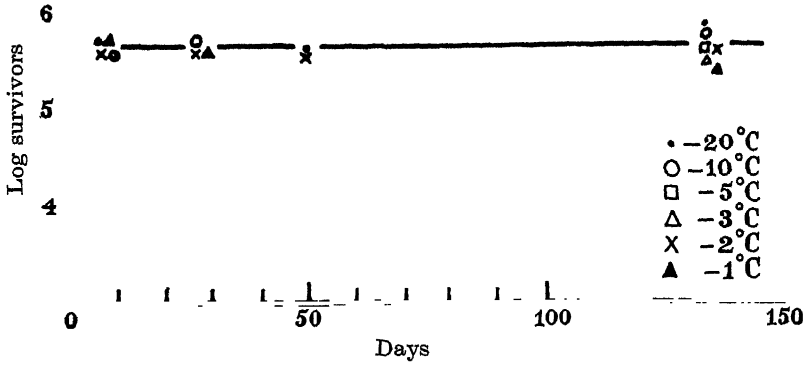

FIG. 4—Death of spores of *B. mesentericus* in frozen suspensions.

TABLE IV—EFFECT OF TRANSFERRING FROZEN SUSPENSION FROM −20 TO −2° C.

Viable organisms per tube. Mean of three

Time in days	29	36
Viable count at −20° C.	893,000	290,000
Mean count on tubes transferred to −2° C. from 29 to 36 days	—	41

It seems clear that there is a rapid rate of death associated characteristically with temperatures near −2° C. In the case of muscle it is possible to fix the critical temperature at −2° C., but in measuring the death of bacteria the errors are so great that it is impossible to differentiate systematically between −1 and −2° C. The rate of death at −1 and −2° C. is, however, on the whole more rapid than at −3° C., and definitely more rapid than at −5° C.

THE EFFECT OF FREEZING ON THE PROTEINS OF *B. PYOCYANEUS*

90 litres of a nutrient broth culture of *B. pyocyaneus*, grown for 3 days at 20° C., were centrifuged, generally in 3–5 l. lots. The mass of bacterial cells from each centrifuging was washed twice in ice-cold water with re-centrifuging. It was then dried by taking through mixtures of alcohol and ether, beginning with absolute alcohol distilled over lime, and finishing with anhydrous ether, after the method of Hardy and Gardiner (1910) as

modified by Gough (1933). The soxhlet extraction with ether as used by Gough for the tubercle bacillus was, however, omitted. The dried cells were finally suspended in anhydrous ether at − 3° C., and the ether removed in vacuo; 35 g. of dried cells were obtained. The mass of dried cells was ground in a ball-mill at − 3° C. for 48 hr. Extraction of the contents of the mill was carried out with ice-cold water, the final bulk being about 1 l. After centrifuging, the liquid was filtered at the pump. All these operations were carried out at 0° C. or lower.

The resulting solution contained heat-coagulable protein, having a nitrogen content as determined by micro-Kjeldahl of 17 mg. for 10 c.c. Positive results were obtained in the following colour reactions for proteins: Mellon, Sakaguchi, Molisch, zanthoproteic, glyoxylic and ninhydrin. To investigate the effects of freezing, 10 c.c. lots were distributed in test-tubes, frozen rapidly at − 70° C., and stored at − 20 and − 2° C. After a few days' storage at − 2° C., a flocculent precipitate settled out of the tubes on thawing. The precipitate gave positive results in the above series of colour reactions for proteins. These results suggest that the precipitate is, in fact, a protein that has undergone denaturation on freezing in a manner analogous to the denaturation of myogen in muscle juice (Finn 1932). It is probably "globulin" in character, using the term "globulin" in the sense of a water-soluble protein precipitated by half saturation with ammonium sulphate, and not a true water-insoluble, salt soluble, globulin. The evidence for the "globulin" character of the precipitate is at present incomplete, but little protein seemed to be taken out of the ground cells by 5 % sodium chloride solution, and most of the protein seemed to be precipitated from aqueous extracts by half saturation with ammonium sulphate, very little coming out on full saturation. It is hoped to investigate this point further.

To obtain quantitative data on the flocculation of protein caused by freezing, a series of tubes of the solution of bacterial extract was rapidly frozen and put down at − 20 and − 2° C. Tubes were withdrawn at intervals of 1, 2, 4, 6, 8 and 12 days, thawed, and allowed to stand for 2 hr. at room temperature, in order to allow any precipitate to settle out. 2 c.c. lots were then carefully removed, without disturbing the precipitate, and the nitrogen content determined by the micro-Kjeldahl technique. Ideally, it would have been preferable to determine the increase of nitrogen in the precipitate, as Finn did with muscle extract, but this was impracticable owing to the small quantities available. The alternative procedure of following the decrease in nitrogen in the solution proved quite feasible. In general, determinations were carried out in quadruplicate, and reasonable agreement was obtained. Six determinations of the zero point on the unfrozen solution, and on the solution frozen and thawed immediately, were made. The total coagulable protein in the unfrozen solution was also determined by precipitation with trichloracetic acid (10 %). The results of the analyses are given in Table V.

TABLE V—CHANGES IN NITROGEN CONTENT OF FROZEN BACTERIAL EXTRACT

Total N in solution = 17·13 ± 0·08 mg. N per 10 c.c.

Non-protein N left after trichloracetic acid precipitation = 4·84 ± 0·22 mg. N per 10 c.c.

Protein N by difference = 12·29 mg. N per 10 c.c.

N in solution frozen and thawed immediately = 17·15 ± 0·44 mg. N per 10 c.c.

	mg. N per 10 c.c. of thawed solution after allowing precipitate to settle	
	Stored at −2° C.	Stored at −20° C.
Days		
0	17·15 ± 0·44	17·15 ± 0·44
1	14·06 ± 0·10	—
2	13·40 ± 0·86	—
4	10·99 ± 0·95	16·65 ± 0·37
8	10·17 ± 0·80	16·78 ± 0·63
12	*11·45	17·01 ± 0·04

* Single observation.

In fig. 5 the increase of the precipitate, expressed as the percentage of the total coagulable protein in the solution, in terms of nitrogen, is plotted against time of storage for the two temperatures −20 and −2° C.

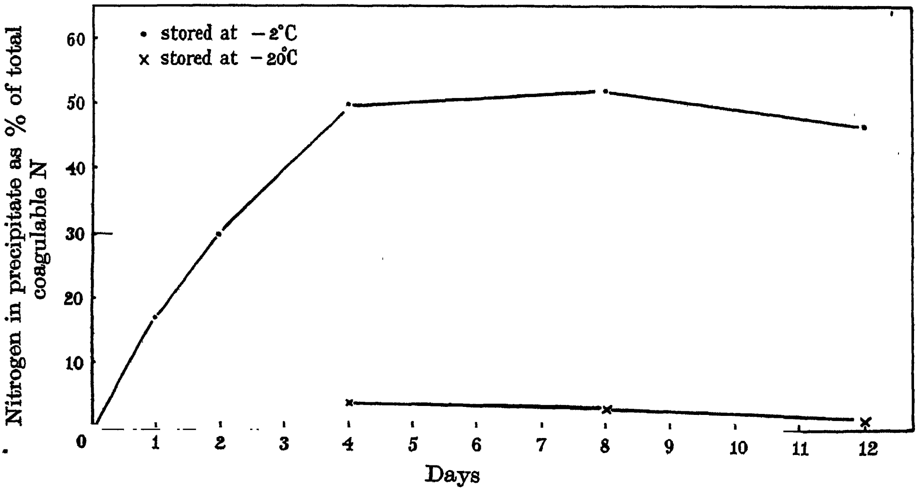

FIG. 5—Flocculation of bacterial protein in frozen solution.

It may be seen that no significant change in the nitrogen content of the solution takes place on rapid freezing and thawing at −70° C. In the tubes stored at −20° C. a very slight decrease in the nitrogen content takes place, but in the tubes kept at −2° C. a rapid accumulation of precipitate occurred, amounting in 1 day to nearly 17 % of the "total coagulable protein nitrogen" in the solution, and in 8 days to about 50 %, after which little further change apparently took place.

COMMENT

Two aspects of the effect of freezing on bacteria can be observed. First, when bacterial suspensions are frozen rapidly at −70° C. and thawed, a certain proportion of the cells is killed, varying from about 80 % with the most susceptible organism, *B. pyocyaneus*, to little or none with spores. The mortality is constant under these conditions with young cells, but varies somewhat with the manner of freezing. The rate or temperature of freezing appears to have little effect. Secondly, when the quickly frozen suspensions are stored at such temperatures as −1, −2, −5, −10 and −20° C., the rate of death again varies with the organism, being most rapid with *B. pyocyaneus* and little or none with spores. With some organisms, notably *B. pyocyaneus* and *B. coli* rapid death occurs near the highest temperature of storage and comparatively slow death at −20° C. When there is any difference in the rate of death between the upper and lower temperatures, it is always in this order.

In attempting to explain these observations, frozen and unfrozen cells were examined by dark ground illumination and in stained preparation. It is often stated that mechanical destruction of bacteria when frozen in bulk can be obtained with repeated freezing and thawing (e.g. Young 1929), but no evidence for its occurrence, either in the single freezing or after storage in the frozen state, could be found under the conditions of the present experiments. Damage to the cellular membrane, leading to sufficiently increased permeability for the liberation of enzymes, might, however, well escape observation. Turning to the effects of freezing on the proteins of the bacterial cell, it was found that rapid flocculation of one protein fraction of an extract of *B. pyocyaneus*, prepared at low temperatures to obtain the "native" proteins, occurred at −2° C. Such flocculation took place slowly, if at all, at −20° C. It seems likely, therefore, that the death of bacteria in the frozen state is due to some change, or complex of changes, in one moiety of the cellular proteins leading to denaturation and subsequent flocculation, owing to the particular salt concentration and *p*H obtained. This observation probably furnishes the explanation of the success of methods of rapid freezing, with desiccation, for the storage of bacteria and sera, since the cellular protein most sensitive to changes in environment can be obtained in an undenatured condition by extraction at low temperatures, and keeps indefinitely when dry.

No flocculation could be demonstrated on a single freezing and thawing, and yet with some organisms a big mortality occurs during this process. A similar phenomenon has, however, been found in muscle (*op. cit.*) and the explanation put forward by Hardy (1928) of the occurrence of a time-lag, may hold here. The picture suggested is that some change is brought about in the organization of the cellular proteins, either denaturation itself or some change leading to denaturation, by the unfavourable environment set up at temperatures just below zero. The proteins, or one fraction, are then subsequently flocculated, and this flocculation can be measured. If the critical zone of temperature is passed through rapidly, less damage is done to the cell. If this view is correct, it suggests that the resistance of spores is due to the fact that their cells do not contain the particular protein most sensitive to changes in environment. This point is under investigation in a more extended study of the proteins of bacteria.

SUMMARY

1—Rapid freezing of aqueous suspensions of bacteria under defined conditions leads to death of a constant proportion of the cells, varying from about 80 % with the most sensitive organism, *B. pyocyaneus*, to little or none with spores.

2—The temperature or rate of freezing appears to have little effect on the mortality within specified limits.

3—Storage of aqueous suspensions of bacteria in the frozen state at temperatures from − 1 to − 20° C. gives a most rapid rate of death near the highest temperature of storage.

4—It is shown that storage at − 2° C. of a frozen solution of the native cellular proteins of *B. pyocyaneus*, extracted at low temperature, leads to rapid coagulation of one fraction of the proteins. Such flocculation is negligible at − 20° C.

5—It is suggested either that two factors are responsible for the death of the bacterial cell on freezing: one unknown, but apparently not mechanical, and the other, some change leading to flocculation of the cellular

proteins; or else that there is in reality one process, leading to coagulation, but with a time-lag, so that coagulation is not found on immediate freezing and thawing.

REFERENCES

Finn, D. B. 1932 *Proc. Roy. Soc.* B, **111**, 396–411.
Fisher, E. A. 1924 *J. Phys. Chem.* **28**, 360–7.
Gough, G. A. C. 1933 *Biochem. J.* **27**, 1049–54.
Hardy, W. B. 1928 *Colloid Symp. Monogr.* **6**, 7–16.
Hardy, W. B. and Gardiner, S. 1910 *J. Physiol.* **40**, 68 *P*.
Knaysi, G. 1930 *J. Bact.* **19**, 113–15.
Lepeschkin, W. W. 1924 "Kolloidchemie des Protoplasmas." Berlin.
MacFadyen, A. and Rowland, S. 1900 *Proc. Roy. Soc.* **66**, 488–9.
Maximov, N. A. 1912 *Ber. dtsch. bot. Ges.* **30**, 52–65, 293–305, 504–16.
Moran, T. 1929 *Proc. Roy. Soc.* B, **105**, 177–97.
— 1935 *Proc. Roy. Soc.* B, **118**, 548–59.
Morrison, T. F. 1924 *J. Gen. Physiol.* **7**, 741–53.
Smith, E. C. 1929 *Proc. Roy. Soc.* B, **105**, 198–207.
Wámoscher, L. 1930 *Z. Hyg. InfektKr.* **111**, 422–60.
Wilson, G. S. 1922 *J. Bact.* **7**, 405–46.
Young, E. G. 1929 *Biochem. J.* **23**, 831–9.
Zsigmondy, R. 1911 *Z. anorg. Chem.* **71**, 356.

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The Suppression of Oestrus in the Rat during Pregnancy and Lactation

By T. McKeown and S. Zuckerman

Department of Human Anatomy, Oxford

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[Plate 18]

It is well known that one function of the corpus luteum is the inhibition of oestrus. This fact has been established by experiments in which corpora lutea are removed and by experiments in which the functional life of corpora is prolonged. More recently it has been shown that the corpora exercise this inhibiting influence by means of their internal secretion. The exact part they play in the suppression of oestrus during pregnancy and in lactation in the rat is, however, still obscure. An attempt was therefore made in the following investigation to study the influence of destruction of luteal tissue on the return of oestrus and on the ripening of follicles, a technique which to our knowledge has not before been applied to the rat.

Animals and methods

The animals used in our experiments were adult female rats of the albino and black and white varieties, whose sexual cycles were followed by daily examination of vaginal smears. The beginning of pregnancy was determined in animals left with normal males by the finding of sperm in the vagina. The beginning of pseudo-pregnancy was dated from the appearance of the last oestrus in animals left with vasectomized males.

Corpora lutea were destroyed as follows. The ovaries were usually approached by dorso-lateral incisions, and their capsules were opened so as to expose all the corpora lutea. An electro-cautery needle was applied to each corpus in turn, a weak current being used, and care being taken that non-luteinized parts of the ovary should not be harmed. The procedure is relatively simple, and the amount of damage done can be gauged by the gradual whitening of the tissue. Parts of the ovaries frequently showed no corpora lutea, and the danger of damage to non-luteal portions was correspondingly reduced. That the operation can be successfully accomplished

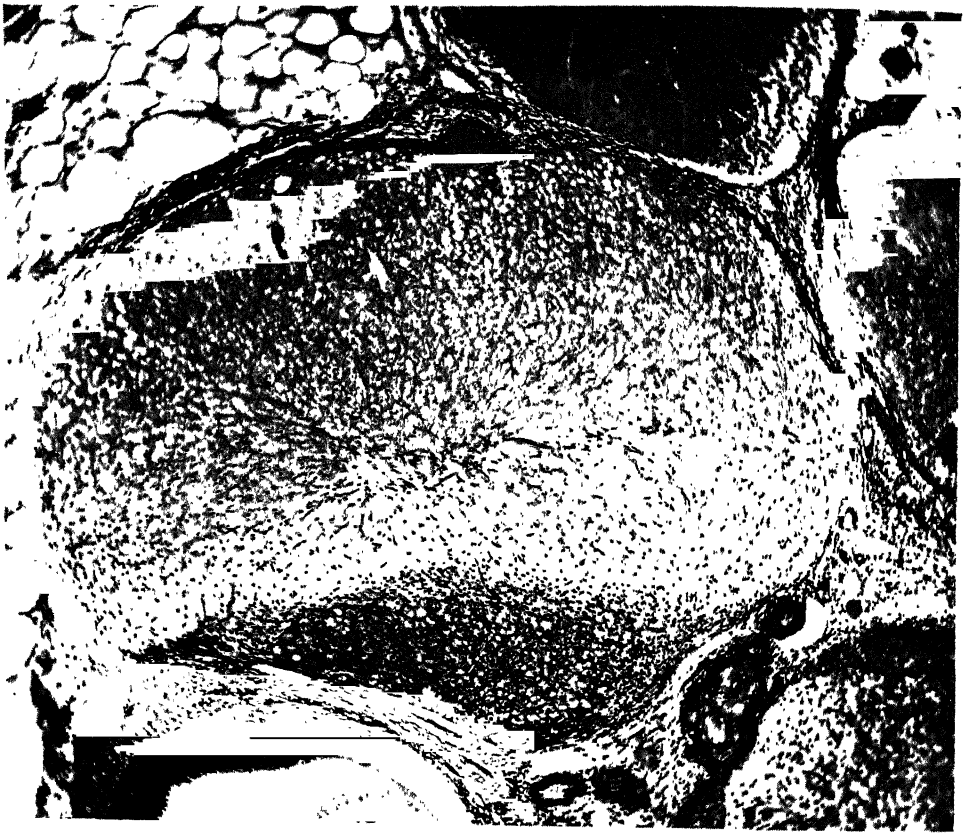

Fig. 1—Corpus luteum after application of electro-cautery needle, showing some healthy cells. × 78.

in the rat is demonstrated by the almost immediate ripening of follicles and by the reappearance of oestrus in animals operated on in pseudo-pregnancy and in early pregnancy.

The ovaries of the experimental animals were serially sectioned, and stained with Ehrlich's haematoxylin and eosin. The rats were autopsied at varying times after operation. In some there had been no post-operative oestrus; others were killed at the time of the first post-operative oestrus; and the remainder were autopsied at the end of the first post-operative cycle.

The uterus of each of the animals that were operated on in the second half of pregnancy was examined histologically at the close of the experiments.

HISTOLOGY

It proved impossible to assess the extent of destruction of luteal tissue. Every ovary showed several corpora which were recognizable as such only because of their general arrangement. Cauterization had transformed them into homogeneous eosinophilic bodies which possessed no cellular structure and which were supplied by widely dilated vessels. Other corpora showed regions in which the original cellular structure could still be recognized (fig. 1, plate 18). Moreover, in all but three animals that were operated on in late pregnancy, one or more deeply embedded corpora lutea, which presumably did not date from the ovulation immediately preceding the operation, appeared to have escaped destruction. Some of them were corpora lutea atretica, and in almost every instance the persisting luteal tissue showed obvious signs of degeneration. It is well known that corpora lutea disappear very slowly from the ovaries of small rodents (Long and Evans 1922). Deanesly (1930) writes: "In the absence of pregnancy old corpora lutea persist for a considerable period in the mouse ovary, and after initial signs of degeneration which are not always obvious, further changes are slow to occur."

While the histological evidence is not conclusive, it is evident from the results of the experiments detailed below that the electro-cautery is in fact an effective way of ending a luteal phase of ovarian activity in the rat.

EXPERIMENTAL

Pseudo-pregnancy

Preliminary experiments were conducted on pseudo-pregnant animals in order to test the method. Seven normal cyclic adult female rats were mated with vasectomized males. The corpora lutea which consequently developed were destroyed at some time between the 3rd and 7th days of pseudo-pregnancy.

All the animals came into pro-oestrus by the 3rd day after operation, and into full oestrus by the 4th day. As Table I shows, the experimental dioestrous period varied between 7 and 11 days. The corresponding period in five control pseudo-pregnancies lasted 12–15 days. These results indicate that the functional luteal tissue had been effectively destroyed. Since a new phase of follicular growth had occurred, it was also plain that the follicular apparatus had not been seriously disturbed by the operative procedure.

TABLE I—THE RETURN OF OESTRUS AFTER DESTRUCTION OF CORPORA LUTEA DURING PSEUDO-PREGNANCY

Animal	Operation	Day of operation (dated from beginning of pseudo-pregnancy)	Day of return of oestrus (dated from beginning of pseudo-pregnancy)
1	Corpora lutea destroyed	7	11
2	,, ,, ,,	6	10
3	,, ,, ,,	6	10
4	,, ,, ,,	6	9–10
5	,, ,, ,,	5	7–8
6	,, ,, ,,	4	7
7	,, ,, ,,	3	7
8	Control—unoperated	—	14
9	,, ,,	—	15
10	,, ,,	—	15
11	,, ,,	—	12
12	,, ,,	—	13

First Half of Pregnancy

The first and second halves of pregnancy have been regarded as separate reproductive phases for the purpose of the present investigation in view of evidence which indicates their different endocrine mechanisms (see Selye and McKeown 1935).

Three adult female rats were operated upon on the 6th and 7th days of pregnancy. All corpora lutea were destroyed, and the foetuses and placentae were removed. Two other pregnant animals were operated upon on the 4th and 5th days of pregnancy. In these the corpora lutea were destroyed but the pregnant uterus was not disturbed.

As Table II shows, oestrus returned within 4 days of the operation in four of these five experimental animals. The fifth animal, from which the corpora lutea, foetuses and placentae had been removed, came into oestrus on the 13th day after the operation.

TABLE II—THE RETURN OF OESTRUS AFTER DESTRUCTION OF CORPORA LUTEA (WITH OR WITHOUT CAESARIAN SECTION) IN EARLY PREGNANCY

Animal	Operation	Day of operation (dated from beginning of pregnancy)	Day of return of oestrus (dated from beginning of pregnancy)
1	Corpora lutea destroyed; placentae and foetuses removed	7	10
2	,, ,, ,,	6	13
3	,, ,, ,,	6	10
4	Corpora lutea destroyed	5	9
5	,, ,, ,,	4	8
6	Placentae and foetuses removed	7	12
7	,, ,, ,,	6	14
8	,, ,, ,,	7	14

Caesarian section performed on the 6th or 7th day of pregnancy does not by itself lead to the return of oestrus until after the 12th day (Selye and McKeown 1935). This observation was confirmed in three experiments (Table II).

In the circumstances it follows that the suppression of oestrus in the first half of pregnancy is immediately related, as in pseudo-pregnancy, to the presence of corpora lutea, and is not dependent on the uterine contents. The results of these experiments show clearly that even if the placentae secrete progestin at all during the first part of gestation, they do not secrete enough to suppress oestrus.

Second Half of Pregnancy

Twelve rats were operated on at some time between the 11th and 16th days of pregnancy, all visible corpora lutea being destroyed. The animals were autopsied from 5 to 8 days after the operations.

Ten of these rats were still in dioestrus at the time of autopsy. The two exceptions aborted soon after the operation, and subsequently came into oestrus, a result which is in keeping with the fact that Caesarian section in the second half of pregnancy in the mouse and the rat is also followed by oestrus (Newton 1935; Selye and others 1935).

TABLE III—DESTRUCTION OF THE CORPORA LUTEA IN LATE PREGNANCY

Animal	Operation	Day of operation (dated from beginning of pregnancy)	Result of operation	Day of autopsy (dated from beginning of pregnancy)	Autopsy and histological findings
1	Corpora lutea destroyed	11	No return of oestrus	18	One foetus alive
2	,, ,,	11	,, ,,	17	Normal pregnancy
3	,, ,,	15	,, ,,	20	Some placentae normal
4	,, ,,	12	,, ,,	18	Three foetuses alive
5	,, ,,	13	,, ,,	19	Some placentae normal
6	,, ,,	12	,, ,,	18	Three foetuses alive
7	,, ,,	11	,, ,,	17	Some placentae normal
8	,, ,,	12	,, ,,	18	1 or 2 foetuses alive
9	,, ,,	11	Oestrus—18th day	19	Aborted
10	,, ,,	11	No return of oestrus	17	Some placentae normal
11	,, ,,	11	,, ,,	17	,, ,,
12	,, ,,	16	Oestrus—18th day	19	Aborted

It would thus seem that the corpora lutea of the second half of pregnancy differ both from those of the first half of gestation and from those of pseudopregnancy in not being the factors essentially responsible for the suppression of oestrus. Our findings indirectly confirm the conclusion which Newton (1935) drew from a different type of experiment. Newton removed the foetuses from pregnant mice between the 12th and 15th days of gestation, being careful to leave the placentae in place, and not to disturb the ovaries. The placentae were subsequently delivered spontaneously at full term, and a post-partum oestrus occurred 1–2 days later. If, however, the placentae were removed at the same time as the foetuses, oestrus occurred significantly sooner than it would have done if the animals had not been disturbed. These experiments suggest, as Newton points out, that the placenta plays an essential part in inhibiting oestrus during the latter half of gestation. There is, of course, no reason to suppose that the actual agent which determines the suppression of oestrus is not progestin, and it is of interest that evidence is available which indicates that the hormone is produced by the placenta (Ehrhardt 1934; Adler, Fremery and Tausk 1934; Selye and others 1935; Ehrhardt and Fischer-Wasels 1936).

Newton's own experiments throw no light on the part played by the corpora lutea in the suppression of oestrus in pregnancy, but his observations make it clear that the reason why oestrus does not normally follow destruction of the corpora in the second half of gestation is because the operation does not normally lead to the death of the placentae.

Though the corpora lutea do not seem to be essential for the inhibition of oestrus in the second half of pregnancy, they appear to exercise considerable influence on the development of the foetuses (see Table III). Thus removal of the corpora lutea in two of the twelve animals resulted in abortion. In five the foetuses were dead (in a few cases in process of resorption) but some placentae appeared to be normal. In four animals some, but not all, foetuses were dead. In only the 12th animal were the uterine contents completely normal.

At first sight these results seem different from those which Parkes, using mice (1928), and Pencharz and Long, using rats (1932), obtained in their study of the role of the corpus luteum in pregnancy, but the difference may be more apparent than real. One ovary of each of the animals used by these investigators was first sterilized by means of X-rays. The animals were later allowed to become pregnant, and at various times after the 8th day of gestation the normal ovary, which contained the corpora lutea of gestation, was removed. It was found that resorption of the foetuses and abortion, as indicated by the presence of "large amounts" of blood in the vaginal smear, always occurred about 2 days after the unilateral ovariectomy.

The essential difference between the results of these experiments and those of our own is that abortion invariably followed removal of the one ovary containing the corpora lutea of pregnancy, whereas total abortion was an atypical result of destruction of the corpora lutea of pregnancy without removal of the ovaries. In view of the uniformity of our results in respect of the return of oestrus (Tables I–III), it is unlikely that the survival of some foetuses and placentae after destruction of the corpora was due to some deficiency in the technique used in destroying the corpora. This belief is supported by the fact, recorded by Selye and others (1935), that the placentae of pregnant rats do not die after ovariectomy. Klein (1933) has also found that the placentae persist after removal of the ovaries of pregnant rabbits. In interpreting the discrepancy between our results and those obtained in irradiation experiments, it may therefore be significant that both Parkes and Pencharz and Long took the presence of "large amounts" of blood in the vaginal smear as indicative of the termination of the pregnant state. They do not, however, refer to the occurrence of oestrus after the presumed abortion, and it is thus not unlikely that the placentae of their animals remained intact after removal of the non-irradiated ovary. On the other hand, it is conceivable, in seeking for an explanation of the apparent difference in our results, that the removal of one normal ovary, even though an irradiated ovary is left undisturbed, removes some factor that is essential to the maternal organism, which is not disturbed by bilateral destruction of the corpora lutea alone.

It is generally believed that removal of both ovaries from normal pregnant rats results in abortion. Haterius (1936) has, however, found that if all but one of the foetuses are killed, the surviving foetus may be carried to term after bilateral ovariectomy of the mother if the placentae are left undisturbed. The healthy growth of the surviving foetus is attributed to the secretion of progestin by the placenta, a view that is supported by the findings of Selye and others (1935), and which is in keeping with the fact that pregnancy can be maintained both in normal rabbits after ovariectomy (Allen and Corner 1930; Courrier and Kehl 1933), and in hypophysectomized rabbits (Robson 1936), by means of progestin. Since the removal of the ovaries of pregnant rats does not affect the life of the placentae, as Selye and his co-workers have found, it becomes possible in the light of Haterius' results to understand why at least one foetus may survive ovariectomy of the mother, as was observed both by Marshall and Jolly (1906) and by Hain (1934), and why ovariectomy carried out late in pregnancy is not always immediately followed by abortion. In this respect the results of destruction of the corpora lutea are also in accord with expectation, for this procedure does not as a rule lead to the death of the placentae, and one or more foetuses usually survive the operation. It is, of course, possible that the effects of ovariectomy in pregnancy differ from those of destruction of the corpora lutea because the well-being of the foetuses is dependent not only on progestin, but on some other ovarian secretion as well.

Regardless of this possibility, the following definite conclusions are indicated by the facts: (*a*) the life of the placentae in the latter part of pregnancy is not wholly dependent on the ovaries; (*b*) the life of the corpora lutea in the latter part of pregnancy is at least partly dependent on the placentae; (*c*) the life of the foetuses is dependent on the progestin produced by the corpora lutea of gestation and possibly by the placentae; (*d*) the amount of progestin produced by the placentae is far less than that provided by the corpora lutea, since the placentae as a rule cannot sustain more than one foetus in the absence of corpora lutea; (*e*) the progestin threshold for the inhibition of oestrus during pregnancy is much lower than that for the maintenance of the foetuses, since oestrus continues to remain in abeyance after destruction of the corpora lutea during the period normal placental tissue survives. The fact that oestrogenic hormone plays a part in the later changes of pregnancy is suggested by the work of Reynolds and Firor (1933) and of Robson (1936).

It may be noted that destruction of the corpora lutea of the rabbit in the latter phase of pregnancy invariably leads to abortion (Klein 1932). In this the rabbit differs from the rat (for other species differences in the physiology of pregnancy see Selye and others 1935; Haterius 1936; and Robson 1936, 1937).

Lactation

The part, if any, played by the corpora lutea in the suppression of oestrus during the lactation interval of the rat and mouse is by no means clear. Parkes and Bellerby (1927) found that much more oestrin is necessary to produce oestrus in lactating mice than in spayed mice, and that the amount required in lactation is roughly proportional to the number of young suckling. Moreover, it was shown that after ovariectomy the amount of hormone required to produce oestrus is little more than is necessary in the ordinary ovariectomized animal. These experiments, which have been confirmed by Robson (1934), suggested that the ovary during lactation has considerable oestrus-inhibiting power, and further investigation on unilaterally X-irradiated mice, in which the normal ovary was removed during lactation, suggested that this effect is exerted solely through the corpora lutea (Parkes 1929*a*). If the corpora are the sole agents responsible for the suppression of oestrus, it follows that they are in some way maintained by the act of suckling, for oestrus will occur within 2–4 days after the removal of the litter—at whatever time the young are removed during the three weeks' lactation interval. From the same premise, it also follows that oestrus should occur after destruction of the corpora lutea in spite of the continuation of lactation. This deduction is not, however, borne out by experiment.

TABLE IV—DESTRUCTION OF THE CORPORA LUTEA IN LACTATION

Animal	Day corpora lutea destroyed (dated from day of delivery)	Date of weaning (dated from day of delivery)	Day of return of oestrus (dated from day of delivery)
1	2	4	9
2	2	3	9
3	2	2	8
4	2	4	9
5	4	6	No oestrus (animal ill)
6	2	9	15
7	2	9	14
8	3	12	18
9	2	Not weaned	No oestrus—killed 8th day
10	2	,, ,,	,, ,, 10th day
11	2	,, ,,	,, ,, 16th day
12	2	,, ,,	,, ,, 21st day

The corpora lutea were destroyed in both ovaries of 12 lactating rats. Five of these animals (Nos. 1–5) were weaned soon after the operation. Of these five, four resumed normal oestrus cycles within 6 days (see Table IV). The fifth animal was ill. Three (6–8) were weaned on the 9th, 9th and 12th days of lactation respectively, and oestrus returned within 6 days of weaning in each of these three animals.

The remaining four of the operated animals (9–12) were left with their litters. Oestrus did not occur while suckling continued, and the animals were killed at varying times during the lactation dioestrus.

Lactation appeared to be normal in animals 6–12, and histological examination showed that the mammary glands were in a fully developed and functional state.

Since oestrus did not occur in spite of the removal of the corpora lutea, it follows that its suppression during lactation cannot be entirely due to these organs.

DISCUSSION

The data considered under the heading *Second Half of Pregnancy* show that the progestin threshold for the suppression of oestrus is relatively low, and that enough of the hormone is elaborated by the placentae to inhibit oestrus even after destruction of the corpora lutea. The results of our experiments on lactating rats suggest that in lactation, too, there must be another factor additional to the corpora lutea (which Parkes has shown to have a powerful oestrus-inhibiting effect during this reproductive phase) which is concerned in the suppression of oestrus. It is unlikely that this other factor is the metabolic drain of suckling, since, as has been found both by Parkes (1926) and by Crew and Mirskaia (1930), foster-mothering, though it may prolong lactation considerably, does not extend the normal quiescent lactation interval. In the circumstances it would seem that this other factor must be hormonal in character, and the problem of interpreting the mechanism of oestrus suppression in the lactating rat becomes much the same as in species in which the lactation interval is not heralded by a post-partum ovulation, e.g. the ferret (see Parkes 1929*b*) and the monkey (Zuckerman 1931). This problem is not greatly illuminated by the available evidence. The lactogenic hormone of the pituitary may itself be the factor concerned, for both Dresel (1935) and Lahr and Riddle (1936) have found that prolactin is able to inhibit oestrus in mice and rats. In view of recent advances in our knowledge of the relationship of the adrenals to the gonads (see, for example, Callow and Parkes 1936), it is also tempting to speculate that the same hypophyseal stimulus which leads to luteinisation of the ovaries stimulates the adrenals to produce a substance with progestin-like properties, and that the amount produced in this way is enough to suppress oestrus even after destruction of the corpora lutea. Alternatively the facts suggest that the act of suckling in some way inhibits the follicle-stimulating powers of the anterior pituitary, while it simultaneously stimulates the production of the luteinizing hormone. If this be the correct interpretation, the failure of oestrus to follow destruction of the corpora lutea of lactation would be due to an already existing suppression of the follicle and oestrin-stimulating hormone of the pituitary, and the oestrus-inhibiting capacity of the corpora lutea of lactation would have to be regarded as a purely potential power. This view receives added plausibility from the fact that oestrogenic hormone inhibits milk secretion (Folley 1936), and that there is no evidence of the presence of the hormone, as judged by a failure of lactation, after destruction of the corpora lutea—the organs which *a priori* would be regarded as inhibiting the hormone's action. It may be added, too, that the capacity of oestrin to inhibit lactation provides an obvious teleological reason for the suppression of the follicle-stimulating powers of the pituitary.

The occurrence of a post-partum oestrus may be attributed to the fact that the corpora lutea of pregnancy cease functioning by the time of parturition, when the placentae are also lost. Lactation, and a free supply of lactogenic hormone, are not fully established on the first day after delivery, so that neither prolactin nor progestin is available either to inhibit the follicle-stimulating hormone of the pituitary or to counteract the effects of oestrin on the accessory organs.

Whether the suppression of oestrus in the lactating rat is due to the inhibition of the follicle-stimulating powers of the pituitary, or to the inhibiting action of prolactin or of progestin, or to all these factors, it is at least plain that they are all primarily dependent on the act of suckling, and that the inverse relation that exists between the number of young suckling, on the one hand, and the ease with which oestrus can be induced experimentally by the injection of oestrin (or the frequency of its spontaneous occurrence), on the other, is a measure of the effectiveness of the stimulus of sucking on the pituitary.

SUMMARY

1—Destruction of the corpora lutea of both ovaries of seven rats by means of an electro-cautery at various times between the 3rd and 7th days of pseudo-pregnancy led to the reappearance of oestrus within 4 days (experimental dioestrus of 7–11 days). The control pseudo-pregnancy in five unoperated animals lasted 12–15 days.

2—The corpora lutea of three pregnant animals were destroyed and the foetuses and placentae were removed on the 6th, 6th and 7th days of gestation respectively. Oestrus occurred 4, 7 and 3 days later respectively. The corpora lutea alone were destroyed in two pregnant animals on the 4th and 5th days of gestation; oestrus reappeared on the 8th and 9th days respectively. In three control operations Caesarian section on the 6th, 7th and 7th days led to the reappearance of oestrus on the 14th, 12th and 14th days respectively.

3—Oestrus did not follow destruction of the corpora lutea of twelve rats which were operated on at various times between the 11th and 16th days of pregnancy. Two of these animals aborted as the result of the operation, and most of the others showed partial resorption of the uterine contents. In only one was pregnancy normal.

4—Oestrus did not occur in seven lactating animals that were left with their litters after destruction of the corpora lutea of both ovaries. Oestrus reappeared within 5 days in five lactating animals that were weaned soon after the same operation.

These facts are discussed in relation to accepted views concerning the inhibition of oestrus during pregnancy and lactation.

REFERENCES

Adler, A. A., Fremery, P. de and Tausk, M. 1934 *Nature, Lond.*, **133**, 293.
Allen, W. M. and Corner, G. W. 1930 *Proc. Soc. exp. Biol., N.Y.*, **27**, 403.
Callow, R. K. and Parkes, A. S. 1936 *J. Physiol.* **87**, 28*P*.
Courrier, R. and Kehl, R. 1933 *C.R. Soc. Biol., Paris*, **114**, 1317.
Crew, F. A. E. and Mirskaia, L. 1930 *Quart. J. exp. Physiol.* **20**, 105.
Deanesly, R. 1930 *Proc. Roy. Soc.* B, **106**, 578.
Dresel, I. 1935 *Science*, **82**, 173.
Ehrhardt, K. 1934 *Münch. med. Wschr.* **81**, 869.
Ehrhardt, K. and Fischer-Wasels, H. 1936 *Mschr. Geburtsh. Gynäk.* **102**, 80.
Folley, J. F. 1936 *Biochem. J.* **30**, 2262.
Hain, A. M. 1934 *Quart. J. Exp. Physiol.* **24**, 101.
Haterius, H. O. 1936 *Amer. J. Physiol.* **114**, 399.
Klein, M. 1932 *C.R. Soc. Biol., Paris*, **109**, 932.
— 1933 *C.R. Soc. Biol., Paris*, **113**, 441.
Lahr, L. and Riddle, O. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **34**, 880.
Long, J. A. and Evans, H. M. 1922 *Mem. Univ. of Calif.* **6**.
Marshall, F. H. A. and Jolly, W. A. 1906 *Philos. Trans.* B, **198**, 99.
Newton, W. H. 1935 *J. Physiol.* **84**, 196.
Parkes, A. S. 1926 *Proc. Roy. Soc.* B, **100**, 151.
— 1928 *J. Physiol.* **65**, 341.
— 1929*a* *Proc. Roy. Soc.* B, **104**, 171.
— 1929*b* "The Internal Secretions of the Ovary." London: Longmans, Green and Co.
Parkes, A. S. and Bellerby, C. W. 1927 *J. Physiol.* **62**, 301.
Pencharz, R. I. and Long, J. A. 1932 *Amer. J. Anat.* **50**, 1.
Reynolds, S. R. M. and Firor, W. M. 1933 *Amer. J. Physiol.* **104**, 331.
Robson, J. M. 1934 *Quart. J. Exp. Physiol.* **24**, 337.
— 1936 *J. Physiol.* **86**, 415.
— 1937 *J Physiol.* **90**, 145.
Selye, H., Collip, J. B. and Thomson, D. L. 1935 *Endocrinology*, **19**, 151.
Selye, H. and McKeown, T. 1935 *Proc. Roy. Soc.* B, **119**, 1.
Zuckerman, S. 1931 *Proc. Zool. Soc. Lond.* p. 593.

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The Effect of Sex Hormones on Lactation in the Rat

By S. J. Folley and S. K. Kon

National Institute for Research in Dairying, University of Reading

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Introduction

The mechanism which secures the onset of full lactation at about the time of parturition has long been under investigation. It was once believed that during the gestation period when the mammary gland is undergoing development, lactation was restrained by some inhibitor, the influence of which was removed at parturition, the stimulus of lactation being regarded as of a negative character, that is, merely the removal of an inhibition. The important discovery of Stricker and Grueter (1928) that the anterior lobe of the hypophysis is the source of a lactogenic hormone, prolactin, which provides a positive lactation stimulus has necessitated modification of the earlier view. It should be emphasized that the influence of the hypophyseal lactogenic hormone must be operative to some extent during pregnancy, since numerous histological and cytological studies (e.g. that of Weatherford (1929) on the rat) have shown that mammary secretion begins in the latter half of pregnancy. This is well illustrated by the fact that in late pregnancy considerable quantities of a secretion containing the typical milk constituents can be obtained, by regular milking, from the udders of cows pregnant for the first time (Asdell 1925). The results of Cutler and Lewis (1933) indicate from another angle that the secretory phase of the lactation cycle begins in mid-pregnancy, since by the complement-fixation reaction they could always detect caseinogen in extracts of the udders of pregnant heifers after the 24th week of gestation, but in no case before the 20th week.

The great increase in the rate of milk secretion which follows parturition, indicates, however, either that during pregnancy some influence is operative which partially inhibits lactation or else that during this period the lactation stimulus is only partial. In agreement with the former alternative is the fact that in the cow pregnancy hastens the normal decline in lactation, the depressing effect of pregnancy coming into evidence at about the 5th month (Hammond and Sanders 1923; Ragsdale, Turner and Brody 1924). It is interesting to recall that in 1926, Gaines and Davidson were of the opinion that the accelerated decline in lactation due to pregnancy was due to the operation of a then hypothetical depressing hormone.

The hypothesis of Gaines and Davidson has its more modern counterpart in that of Nelson (see Nelson 1936), who on the basis of extensive experiments has advanced the theory that lactation is inhibited during pregnancy by the oestrogenic hormones of the ovary and placenta, the concentration of which in the blood, as is evident from the work of Ascheim and Zondek (1927) and Nibler and Turner (1929) on the urinary excretion of oestrogenic hormones during pregnancy, progressively increases during the gestation period. According to this theory, with the fall in the blood level of the oestrogenic hormones which occurs at parturition the inhibition is removed and lactation begins. The inhibition is visualized as operating (*a*) by inhibiting the secretion of the lactogenic hormone by the anterior pituitary, and possibly (*b*) by a direct action on the mammary gland. In support of this theory may be cited the considerable amount of evidence which exists to show that lactation is inhibited by administration of oestrogenic hormones. This has been demonstrated by Parkes and Bellerby (1926–7), de Jongh (1933) and Robson (1935) in the mouse, Hain (1935) in the rat, Smith and Smith (1933) in the rabbit, Nelson (1934) in the guinea-pig, and Folley (1936) and Waterman, Freud and Vos-de-Jongh (1936) in the cow.

Anselmino and Hoffmann (1936) claim that while lactation, as judged by the growth rates of suckling young, is inhibited in intact lactating rats by daily injections of oestrogenic hormones, no inhibition is induced by administration of similar daily doses to rats ovariectomized shortly after parturition. They have, therefore, put forward the view that the effect of oestrogenic hormones on lactation is indirect and, in fact, by way of the ovary, the real inhibitor being a substance produced by the corpora lutea, the formation of which is promoted by the oestrogenic hormones. In this connexion it may be noted that Fevold, Hisaw and Greep (1936) report an increase in the luteinizing power of the anterior pituitary following administration of oestrogenic hormones, while Hohlweg (1934), Selye, Collip and Thomson (1935*a*) and others have found that formation of large numbers of corpora lutea is promoted in the ovaries of non-pregnant rats by treatment with oestrogenic hormone. De Jongh (1933), however, reported results quite opposite from those of Anselmino and Hoffmann. Working with mice, he reported inhibition of lactation following oestrogenic hormone administration both in intact and in two cases, ovariectomized animals, while ovariectomized controls which received injections of the oil used for dissolving the oestrogenic hormone were able successfully to rear their young. De Jongh concluded from his experiments that the corpus luteum does not inhibit lactation.

Nelson's theory does not exclude the possibility that the corpus luteum may contribute to the inhibition of lactation during pregnancy. A certain amount of evidence exists which suggests that this may be the case. Thus Drummond-Robinson and Asdell (1926) found that ablation of the corpora lutea in the pregnant goat was quickly followed by milk secretion provided the operation was performed sufficiently late in pregnancy for the mammary lobule-alveolar system to have developed. The experiments of Hammond (1917) point to a similar conclusion. Donahue (1934) brought about luteinization of the ovaries of multiparous rabbits by injections of "anterior pituitary-like" hormone. Development of the mammary glands occurred and milk secretion eventually set in. Laparotomy at the onset of lactation established the fact that the corpora lutea had degenerated. Further, Selye, Collip and Thomson (1933) reported that removal from non-pregnant rats of the luteinized ovaries resulting from injections of "anterior pituitary-like" hormone resulted in milk secretion provided the hypophysis was intact. Anselmino and Hoffmann (1936), however, are of the opinion that though a lactation inhibitor is present in corpus luteum extracts it is not the hormone which induces endometrial proliferation, since, though inhibition of lactation in rats was caused by injections of relatively crude corpus luteum extracts, more purified preparations exerted no inhibitory effect.

In view of the radical disagreement between the results of Anselmino and Hoffmann (1936) and of de Jongh (1933) in respect of the effect of oestrogens on lactation in gonadectomized animals, it was decided to re-examine this question in the light of the possibility that while the dosage of oestrogenic hormone used by Anselmino and Hoffmann might be just sufficient to inhibit lactation in the presence of the ovary, a somewhat higher dosage might be necessary to induce inhibition in its absence. At the same time, the availability of pure crystalline progesterone provided an opportunity of determining directly whether or not this hormone is an inhibitor of lactation.

Recent work on androgenic substances has shown that many androgenic substances in relatively high doses are capable of affecting the female accessory reproductive organs in a similar manner to the female hormones (see Koch 1937 and Marrian and Butler 1937). In particular, testosterone, in addition to causing opening of the vagina in the immature rat (Deansley and Parkes 1936) and mucification in the ovariectomized adult rat, has been shown by Selye, McEuen and Collip (1936) to be capable of causing growth of the mammary ducts in normal and gonadectomized male and female rats. Since testosterone can thus affect the mammary gland in a like manner to oestrogenic hormones, the role of which in promoting the growth of the mammary ducts has been established by numerous workers (see Turner 1932; Nelson 1936), it was considered of interest to compare the action of testosterone on lactation with that of oestrogenic hormones. In order to provide an interesting comparison it was decided to include androsterone in the investigation, since from the work of Deansley and Parkes (1936) and others, androsterone appears to possess little gynaecogenic activity. Recently, Robson (1937), in a preliminary communication, has reported that testosterone propionate causes a definite inhibition of lactation in the mouse, while androsterone has little if any such action.

EXPERIMENTAL

Experimental Animals and Method of Studying Lactation—Female rats from the colony maintained in this Institute were used for this work. The colony consists of hooded Norway rats and has been in existence for seven years, during which time it has been kept under uniform conditions of housing and diet.

In this work the chief criterion of the efficiency of lactation was provided by the slopes of the growth curves of sucklings, additional information on this point being given by changes in the weight of the mothers (since a loss in body weight under the conditions of these experiments probably indicates involution of mammary tissue) and also by the number of deaths (from starvation) among the young. Within 24 hr. of parturition the litters, if larger, were reduced to eight, an even distribution between the sexes being maintained where possible. In cases where a number of rats littered on the same day it was possible to bring small litters up to eight by the transfer of young from larger litters. The indirect method of studying lactation adopted in these experiments proved satisfactory, since, until the eyes of the young open at about the 14th to 16th day post-partum, the latter do not partake of the food of the mother. By that time the results were of such a definite nature that it was unnecessary to feed the mothers in cages separate from their young as recommended by Kozlowska, McCay and Maynard (1932). The combined young of each sex in each litter were weighed each morning from the beginning of the experiment to weaning at the 21st day post-partum, at approximately the same time.

For each treatment groups of four rats were used wherever possible. Table I shows the age of each of the experimental rats together with the number of lactations undergone including the lactation studied.

TABLE I

Group no.	Age of mothers months	Number of lactation
1	10, 10, 10, 10	2, 2, 3, 3
2	10, 10, 10	2, 2, 2
3	10, 10, 10, 10	2, 3, 2, 3
4	10, 10, 10, 10	2, 3, 3, 3
5	13, 13, 10, 10, 10, 10, 10	3, 4, 3, 3, 4, 2, 3
6	13, 10, 10, 10	3, 3, 3, 2
7	10, 10, 10, 10	3, 3, 2, 2
8	10, 10, 10	3, 3, 2
9	8, 8, 8, 8	2, 2, 2, 1
10	8, 8, 8, 8	1, 2, 2, 1

In the experiments on gonadectomized rats the animals were ovariectomized by the dorsal route under Avertin anaesthesia (1 ml. of 2½% Avertin/100 g. body weight intraperitoneally) on the day following parturition.

Injections—The substances used were oestradiol monobenzoate, progesterone, testosterone propionate and androsterone. The first-named substance was actually a mixture of isomerides differing from each other by the stereochemical configuration of carbon atom 17, 6·7 mg. of which are equivalent in oestrogenic activity to 5 mg. of the international standard of oestradiol monobenzoate. The hormones were injected subcutaneously in solution in sesame oil with the exception of progesterone which was injected intramuscularly in arachis oil. In some experiments twelve daily injections were given beginning on the 5th day post-partum, while in the remainder of the experiments the daily injections began on the day following parturition and continued until the 20th day post-partum, i.e. the day before weaning. All experiments included control rats injected with sesame oil. The experiments were so arranged that the daily volume of oil or solution of hormone in oil was always 0·2 ml.

RESULTS

Comparison of the Effects of Oestradiol Monobenzoate, Testosterone Propionate and Androsterone on Lactation in the Intact Rat—In all the experiments dealt with in this section daily injections were begun on the 5th day post-partum, and continued until the 16th day. In Table II are given data relating to the four groups of rats used in this experiment.

TABLE II

Group no.	Treatment	No. of rats in group	Total no. of young at beginning of experiment ♂	♀	No. of young which died during experiment ♂	♀	Change in weight of mothers from beginning of injections to weaning g.
1	0·2 ml. sesame oil daily	4	15	15	0	0	+14, +10, −1, −14
2	Equivalent of 1 mg. oestradiol monobenzoate daily	3	8	15	8	12	−18, −49, −59
3	1 mg. testosterone as propionate daily	4	11	17	0	0	+23, +17, +8, +5
4	1 mg. androsterone daily	4	18	11	0	0	+30, +9, +5, +5

In fig. 1 are shown the average growth curves for the pooled litters of the groups of rats receiving oestradiol monobenzoate, testosterone propionate and sesame oil.

The growth curves in fig. 1 show quite clearly the strongly inhibitory effect of oestradiol monobenzoate in high dosage on lactation in intact rats. This result is in general agreement with the earlier work referred to above. It is also evident from the curves that testosterone propionate in the dosage given, which is relatively high for rats weighing about 300 g., exerts a marked inhibition upon lactation as was found by Robson (1937) to be the case for mice. Under the conditions of this experiment, however, it is clear that the effect is not so marked as the inhibition due to administration of oestradiol monobenzoate. This is brought out not only by the difference in the slopes of the respective growth curves, but also strikingly by the fact that while nearly 90 % of the young of the oestradiol monobenzoate injected rats died before weaning, the rats receiving testosterone propionate were able to rear all their young.

Examination of column 6 of Table II shows that the rats receiving injections of oestradiol monobenzoate showed considerable losses in weight during the course of injections, though by contrast the rats receiving testosterone propionate all gained weight. Halpern and D'Amour (1936) found that continued injections of lower doses of oestrogenic hormones than were given in the above experiments did not cause very marked changes in body weight in the rat. Zondek (1936) on the contrary observed loss of body weight in male rats given oestrogenic hormone injections. In our experiments it is

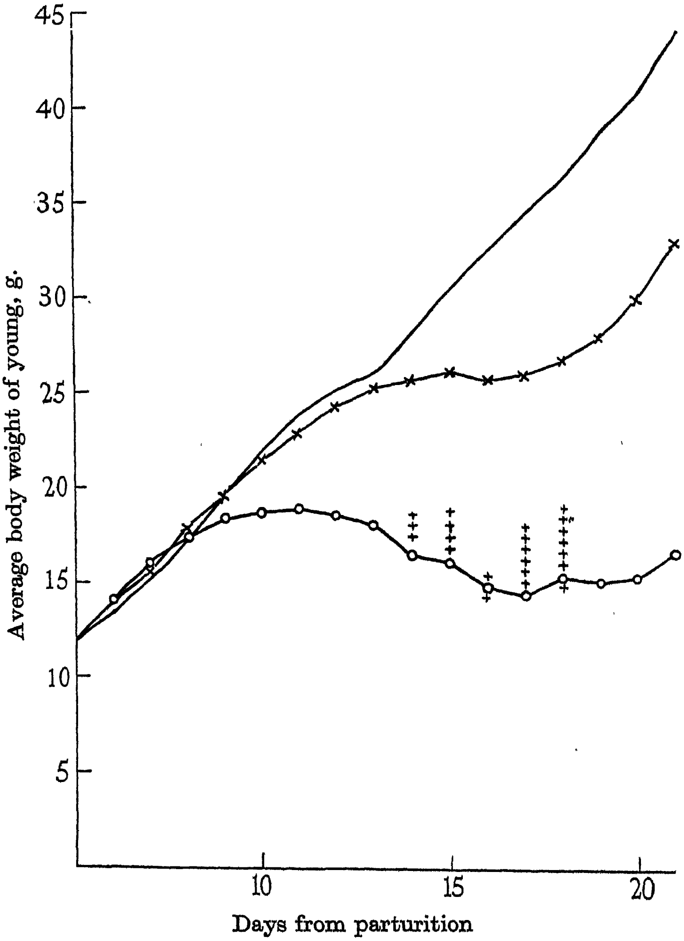

FIG. 1—Average growth curves for pooled litters.

——— Mothers injected with 0·2 ml. sesame oil daily from the 5th to the 16th day post-partum.

×——× Mothers injected with 1 mg. of testosterone as propionate in 0·2 ml. sesame oil daily from the 5th to the 16th day post-partum.

o——o Mothers injected with the equivalent of 1 mg oestradiol monobenzoate in 0·2 ml. sesame oil daily from the 5th to the 16th day post-partum.

+ signifies the death of one member of the litters of mothers receiving oestradiol monobenzoate.

quite probable that some, at any rate, of the marked loss in body weight shown by the rats injected with oestradiol monobenzoate is largely due to the mammary gland involution which must accompany the inhibition of lactation.

The curve for the rats injected with androsterone is for clarity shown together with that for the controls in a separate diagram, fig. 2. It is interesting to find that androsterone, an androgenic substance which does not

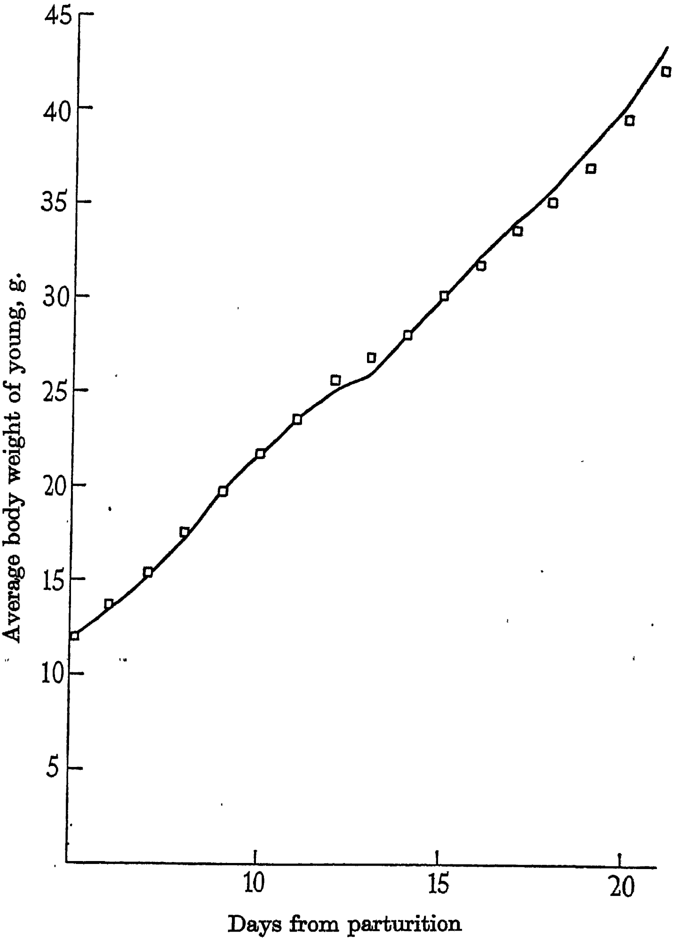

FIG. 2—Average growth curves for pooled litters.

——— Mothers injected with 0·2 ml. sesame oil daily from the 5th to the 16th day post-partum.

□ □ Mothers injected with 1 mg. androsterone in 0·2 ml. sesame oil daily from the 5th to the 16th day post-partum.

appear to possess such strongly marked gynaecogenic properties as a number of other androgens, exerted no inhibitory action on lactation in the high dosage given.

Comparison of the Effect of Oestradiol Monobenzoate on Lactation in the Intact and Ovariectomized Rat—In these experiments daily injections began on the day following parturition and continued until the 20th day post-partum. The rats comprising the gonadectomized groups each received its first injection immediately following ovariectomy. Relevant data are given in Table III.

TABLE III

Group no.	Treatment	No. of rats in group	Total no. of young at beginning of experiment		No. of young which died during experiment		Change in weight of mothers from beginning of injections to weaning g.
			♂	♀	♂	♀	
5	0·2 ml. sesame oil daily (not ovariectomized)	7	29	25	2	0	+24, +23, +20, +12, +7, +6, +1
6	0·2 ml. sesame oil daily (ovariectomized)	4	15	15	1	0	+25, +16, −3, −4
7	Equivalent of 1 mg. oestradiol monobenzoate daily (not ovariectomized)	4	15	13	8	9	−28, −40, −44, −64
8	Equivalent of 1 mg. oestradiol monobenzoate daily (ovariectomized)	3	11	12	0	0	−40, −44, −63

The average growth curves for the combined young of each group are shown in fig. 3.

Comparison of the two curves relating to the groups of rats receiving sesame oil and also of the curves relating to the two groups of rats receiving oestradiol monobenzoate shows that the operation caused a temporary check in lactation. This check was, however, very short-lived and less serious than might have been expected. The results clearly indicate that administration of high daily doses of oestrogenic hormone to lactating rats causes inhibition of lactation in the absence of the ovary as well as in its presence.

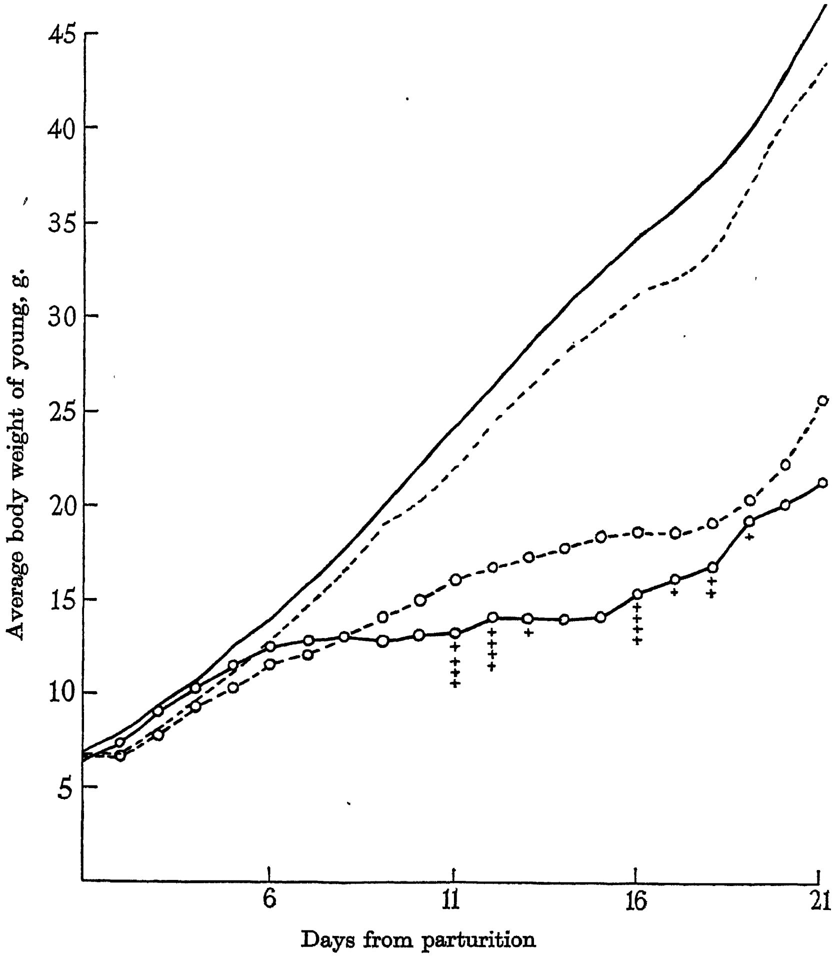

FIG. 3—Average growth curves for pooled litters.

——— Mothers injected with 0·2 ml. sesame oil daily from the 1st to the 21st day post-partum.

- - - - - - Ovariectomized mothers injected with 0·2 ml. sesame oil daily from the 1st to the 21st day post-partum.

o——o Mothers injected with the equivalent of 1 mg. oestradiol monobenzoate in 0·2 ml. sesame oil daily from the 1st to the 21st day post-partum.

o - - - - o Ovariectomized mothers injected with the equivalent of 1 mg. oestradiol monobenzoate in 0·2 ml. sesame oil daily from the 1st to the 21st day post-partum.

+ signifies the death of one member of the litters of mothers receiving oestradiol monobenzoate.

What is most interesting, however, is that with equal daily doses of oestrogenic hormone the inhibition is much less marked in ovariectomized animals than in those with intact ovaries. This is brought out by the difference in the slopes of the growth curves for the two groups and also by the figures given in Table III which show that while about 60 % of the young of the non-ovariectomized rats treated with oestrogenic hormone died during the experiment, none of a total of thirty young of the ovariectomized group died before weaning. The following considerations further emphasize this point. Comparison of the growth curves corresponding to the two groups of rats receiving sesame oil injections shows that, due to the effects of the operation on milk secretion, the young of the ovariectomized mothers are throughout the experimental period lighter in weight than the young of the non-operated rats. A similar effect is initially shown by the growth curves for the two groups of rats receiving oestradiol monobenzoate, but in spite of this initial disadvantage the young of the ovariectomized group soon surpass those of the unoperated group in body weight. Further, as the litters of the intact rats receiving oestradiol monobenzoate become progressively reduced by deaths, the proportion of milk available for each survivor increases. In addition, the surviving young are presumably the most vigorous, so that on both counts reduction of the litters by deaths should result in accelerated growth of the survivors as indeed inspection of the curves shows to be the case. Even so, however, the average body weight of the young of the ovariectomized rats receiving oestradiol monobenzoate is greater at weaning than that of the young of the intact mothers.

In ovariectomized rats, therefore, lactation is much less sensitive to the inhibitory influence of injected oestrogenic hormones than in the case of those with intact ovaries, and it may be inferred that in order to obtain an equal degree of inhibition in the two cases, a considerably larger daily dose is necessary for ovariectomized rats than for intact ones. It therefore seems justifiable to conclude that the dosage of oestrogenic hormone used by Anselmino and Hoffmann (1936), while sufficient to inhibit lactation in the presence of the ovary, fell short of that necessary in its absence.

The marked decrease in body weight in the two groups of rats receiving oestrogenic hormone in contrast to the almost uniform increases in the other two groups is noteworthy.

*The Effect of Progesterone on Lactation in the Rat**—Two groups of four rats were used in this experiment, daily intramuscular injections beginning on the 5th day from parturition and ending on the 16th. The data relating to the experimental animals are given in Table IV.

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* A preliminary account of this experiment was published as a letter in *Nature* (Folley and Kon 1937).

TABLE IV

Group no.	Treatment	No. of rats in group	Total no. of young at beginning of experiment		No. of young which died during experiment		Change in weight of mothers from beginning of injections to weaning
			♂	♀	♂	♀	g.
9	0·2 ml. sesame oil daily	4	14	15	0	1	+12, +9, +7, +6
10	1 mg. progesterone daily	4	17	17	0	1	+24, +18, +15, +12

Average growth curves for the total young of the two groups of rats are plotted in fig. 4.

These results provide no evidence whatever that progesterone given in quite high dosage causes inhibition of lactation. In this respect this experiment supports the contention of Anselmino and Hoffmann (1936) that the hormone which causes uterine endometrial proliferation does not inhibit lactation. It is equally clear from these results that progesterone has no galactogogue action under the conditions of these experiments. Frei and Grüter (1929) reported that injections of corpus luteum extract exerted a favourable effect on lactation in the cow, but the results of our experiments indicate that, unless the reaction of the cow is in this respect different from that of the rat, the galactogogue concerned in Frei and Grüter's experiments could not have been progesterone.

DISCUSSION

In spite of evidence from such experiments as those of Hammond (1917) and Donahue (1934) on the rabbit, Drummond-Robinson and Asdell (1926) on the goat and of Selye, Collip and Thomson (1933) on the rat, that removal of luteal influence is followed by the onset of lactation, the experiments described in this paper make it clear that in the rat, at any rate, progesterone has no inhibitory effect on established lactation. Since the oestrogenic hormones of the ovary and placenta strongly inhibit lactation even in absence of the corpus luteum it is unnecessary to assume, as did Anselmino and Hoffmann (1936), that the corpus luteum, in addition to secreting progesterone, is the source of another substance which has the property of inhibiting lactation.

It is interesting to note that the power of producing development of mammary ducts appears to go hand in hand with the ability to inhibit established lactation. Thus in addition to the follicular hormones oestrone, oestriol and oestradiol which as is well known possess both the above-mentioned physiological activities, testosterone, which was found by Selye, McEuen and Collip (1936) to cause mammary growth in the rat, is also an inhibitor of lactation, while androsterone, which according to Nelson and Gallagher (1936) does not produce mammary development, has no inhibitory

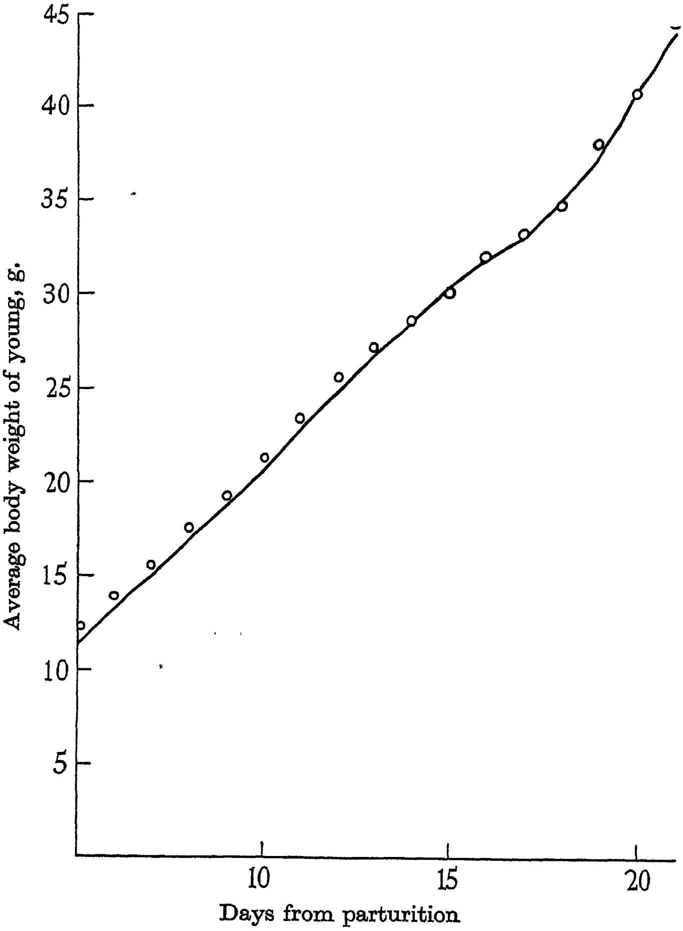

FIG. 4—Average growth curves for pooled litters.

—— Mothers injected with 0·2 ml. sesame oil daily from the 5th to the 16th day post-partum.

o o Mothers injected with 1 mg. progesterone dissolved in 0·2 ml. arachis oil daily from the 5th to the 16th day post-partum.

action on lactation. Further, progesterone, unless administered simultaneously with oestrogenic hormone to animals previously sensitized by oestrogenic hormone administration, causes no mammary growth (Turner and Schultze 1931; Turner and Frank 1932), neither does it, as our experiments show, inhibit lactation. It would be interesting to determine whether or not the simultaneous administration of progesterone and oestrogenic hormone would augment the inhibitory effect of the latter on lactation. The results which appear in fig. 3 indicate that this might be the case, since the decreased sensitivity of spayed rats to lactation inhibition by oestradiol monobenzoate might be due not to the loss of follicular influence but rather to the loss of the corpora lutea. Experiments on this point are projected. If they did prove that progesterone, while possessing no power of inhibiting lactation itself, was capable of augmenting the inhibitory action of oestrogenic hormone, the findings of Hammond (1917), Drummond-Robinson and Asdell (1926) and Donahue (1934) would also become more intelligible.

The mechanism by which the inhibitory effect of oestrogenic hormones operates is at present largely a matter of speculation. Nelson (1936) quotes evidence obtained by him which indicates that oestrogens inhibit lactation by suppressing the secretion of the lactogenic hormone by the hypophysis. Reece and Turner (1937), however, claim that oestrogens do not suppress the secretion of the hypophyseal lactogenic hormone during pregnancy, since the lactogenic hormone content of the pituitaries of pregnant rats was estimated by them to be lower than that of oestrous females, while injection of oestrogens into spayed female rats increased the lactogenic hormone content of their pituitaries over that of controls.

Folley and White (1937) have shown that the enlargement of the pigeon crop gland due to prolactin injections, a physiological response which was discovered by Riddle and Braucher (1931), can be considerably inhibited by simultaneous administration of oestrogenic hormones. This, at first sight, would seem to provide independent evidence that oestrogens inhibit lactation by virtue of their ability, partially at any rate, to "antagonize" prolactin. Actually the results of Folley and White do not provide conclusive evidence of this however, since there is reason to believe that the pituitary is involved in the response of the pigeon crop gland to prolactin. Gomez and Turner (1936*a*) claimed that prolactin has no effect on the crop gland of the hypophysectomized pigeon, while later, Schooley, Riddle and Bates (1937) found that while hypophysectomy does not entirely destroy the crop gland response to prolactin it partially inhibits it.

In the light of what has been said above, it is very possible that the problem of whether or not oestrogenic substances inhibit lactation by way of the pituitary is bound up with the question of the path by which they promote growth of the mammary gland. The position with regard to this is at present far from clear. Some workers (Ruinen 1932; Asdell and Seidenstein 1935; Freud and de Jongh 1935; and Nelson 1935) have been able experimentally to induce mammary growth in hypophysectomized animals by treatment with ovarian hormones, while, on the other hand, Selye, Collip and Thomson (1935*b*), Reece, Turner and Hill (1936) and Gomez and Turner (1936*b*) were unable to do so. Gomez, Turner, Gardner and Hill (1937) point out that mammary growth may be stimulated in "hypophysectomized" male mice if pituitary fragments remain from the operation. This may possibly provide an explanation of the above disagreement. Gomez, Turner and Reece (1937) claim that mammary growth can be stimulated in completely hypophysectomized male guinea-pigs by implantation of pituitaries from donor rats which have been given injections of oestrogenic hormones, while implantation of pituitaries from control rats fails to stimulate mammary growth. This work opens up the possibility that under the influence of oestrogenic hormones the hypophysis secretes a substance, other than prolactin, which is capable of stimulating mammary growth and at the same time of inhibiting lactation. If this possibility were proved true it would provide an explanation for the puzzling results of Corner (1930) who caused mammary development in gonadectomized female rabbits which had never been in oestrus, by injections of anterior pituitary extract.

It is a pleasure to acknowledge our indebtedness to the following for generous gifts of material: Dr A. N. Macbeth of Organon Laboratories for progesterone, Dr K. Miescher of Ciba, Ltd., for testosterone propionate and Dr H. Neumann of Schering, Ltd., for Progynon B Oleosum Forte.

SUMMARY

1—Administration of oestradiol monobenzoate (approximately 0·3 mg. per 100 g. body weight daily) to intact lactating rats causes a marked inhibition of lactation.

2—In rats ovariectomized on the day following parturition, the inhibitory effect of similar doses of oestradiol monobenzoate, while definite, was much less marked.

3—Testosterone propionate (approximately 0·4 mg. per 100 g. body weight daily) administered to lactating rats also inhibited lactation, while progesterone and androsterone in doses of approximately 0·3 mg. per 100 g. body weight daily had no effect on lactation.

4—Rats ovariectomized on the day following parturition and then given daily injections of sesame oil lactated satisfactorily.

5—The bearing of these results on the theoretical aspect of lactation is discussed.

REFERENCES

Anselmino, K. J. and Hoffmann, F. 1936 *Zbl. ges. Gynäk. Geburtsh.* **60**, 501–7.
Ascheim, S. and Zondek, B. 1927 *Klin. Wschr.* **6**, 1322.
Asdell, S. A. 1925 *J. Agric. Sci.* **15**, 358–74.
Asdell, S. A. and Seidenstein, H. E. 1935 *Proc. Soc. Exp. Biol., N.Y.*, **32**, 931–3.
Corner, G. W. 1930 *Amer. J. Physiol.* **95**, 43–55.
Cutler, O. I. and Lewis, J. H. 1933 *Amer. J. Physiol.* **103**, 643–6.
Deansley, R. and Parkes, A. S. 1936 *Brit. Med. J.* **1**, 257–8.
Donahue, J. K. 1934 *Physiol. Zoöl.* **7**, 479–92.
Drummond-Robinson, G. and Asdell, S. A. 1926 *J. Physiol.* **61**, 608–14.
Fevold, H. L., Hisaw, F. L. and Greep, R. 1936 *Amer. J. Physiol.* **114**, 508–13.
Folley, S. J. 1936 *Biochem. J.* **30**, 2262–72.
Folley, S. J. and Kon, S. K. 1937 *Nature, Lond.*, **139**, 1107.
Folley, S. J. and White, P. 1937 *Nature, Lond.*, **140**, 505.
Frei, W. and Grüter, F. 1929 *Virchows Arch.* **275**, 638–44.
Freud, J. and Jongh, S. E. de 1935 *Acta brev. neerl. Physiol.* **5**, 47–8.
Gaines, W. L. and Davidson, F. A. 1926 *Bull. Ill. Agric. Exp. Sta.* No. 272, 1–36.
Gomez, E. T. and Turner, C. W. 1936*a* *Proc. Soc. Exp. Biol., N.Y.*, **35**, 59.
— — 1936*b* *Proc. Soc. Exp. Biol., N.Y.*, **34**, 320–2.
Gomez, E. T., Turner, C. W., Gardner, W. U. and Hill, R. T. 1937 *Proc. Soc. Exp. Biol., N.Y.*, **36**, 287–90.
Gomez, E. T., Turner, C. W. and Reece, R. P. 1937 *Proc. Soc. Exp. Biol., N.Y.*, **36**, 286.
Hain, A. M. 1935 *Quart. J. Exp. Physiol.* **25**, 303–13.
Halpern, S. R. and D'Amour, F. E. 1936 *Amer. J. Physiol.* **115**, 229–38.
Hammond, J. 1917 *Proc. Roy. Soc.* B, **89**, 534–45.
Hammond, J. and Sanders, H. G. 1923 *J. Agric. Sci.* **13**, 74–119.
Hohlweg, W. 1934 *Klin. Wschr.* **13**, 92–5.
Jongh, S. E. de 1933 *Acta brev. neerl. Physiol.* **3**, 52–3.
Koch, F. C. 1937 *Physiol. Rev.* **17**, 153–238.
Kozlowska, M., McCay, C. M. and Maynard, L. A. 1932 *J. Nutrit.* **5**, 61–7.
Marrian, G. F. and Butler, G. C. 1937 *Ann. Rev. Biochem.* **6**, 303–34.
Nelson, W. O. 1934 *Endocrinology*, **18**, 33–46.
— 1935 *Proc. Soc. Exp. Biol., N.Y.*, **33**, 222–4.
— 1936 *Physiol. Rev.* **16**, 488–526.
Nelson, W. O. and Gallagher, T. F. 1936 *Science*, **84**, 230–2.
Nibler, C. W. and Turner, C. W. 1929 *J. Dairy Sci.* **12**, 491–506.
Parkes, A. S. and Bellerby, C. W. 1926–7 *J. Physiol.* **62**, 301–14.
Ragsdale, A. C., Turner, C. W. and Brody, S. 1924 *J. Dairy Sci.* **7**, 24–30.
Reece, R. P. and Turner, C. W. 1937 *Proc. Soc. Exp. Biol., N.Y.*, **36**, 283–5.
Reece, R. P., Turner, C. W. and Hill, R. T. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **34**, 204–7.
Riddle, O. and Braucher, P. F. 1931 *Amer. J. Physiol.* **97**, 617–25.

Robson, J. M. 1935 *Quart. J. Exp. Physiol.* **24**, 337–44.
— 1937 *Proc. Soc. Exp. Biol., N.Y.*, **36**, 153.
Ruinen, F. H. 1932 *Acta brev. neerl Physiol.* **2**, 161–2.
Schooley, J. P., Riddle, O. and Bates, R. W. 1937 *Proc. Soc. Exp. Biol., N.Y.*, **36**, 408–11.
Selye, H., Collip, J. B. and Thomson, D. L. 1933 *Proc. Soc. Exp. Biol., N.Y.*, **30**, 588–9.
— — — 1935*a* *Proc. Soc. Exp. Biol., N.Y.*, **32**, 1377–81.
— — — 1935*b* *Endocrinology*, **19**, 151–9.
Selye, H., McEuen, C. S. and Collip, J. B. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **34**, 201–3.
Smith, G. van S. and Smith, O. W. 1933 *Amer. J. Physiol.* **103**, 356–61.
Stricker, P. and Grueter, F. 1928 *C.R. Soc. Biol., Paris*, **99**, 1978–80.
Turner, C. W. 1932 "Sex and Internal Secretions", ed. E. Allen. Baltimore: Williams and Wilkins. Chap. XII.
Turner, C. W. and Frank, A. H. 1932 *Res. Bull. Mo. Agr. Exp. Sta.* No. 174, 1–28
Turner, C. W. and Schultze, A. B. 1931 *Res. Bull. Mo. Agr. Exp. Sta.* No. 157, 1–45.
Waterman, L., Freud, J. and Vos-de-Jongh, N. 1936 *Acta brev. neerl. Physiol.* **6**, 84–5.
Weatherford, H. L. 1929 *Amer. J. Anat.* **44**, 199–281.
Zondek, B. 1936 *Lancet*, **230**, 776–8.

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Pro-gonadotropic Sera

By I. W. Rowlands

From the National Institute for Medical Research, Hampstead, N.W. 3

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In examining the interaction of a large number of gonadotropic extracts and antigonadotropic sera (Rowlands 1937), two cases were observed where the serum of animals chronically injected with gonadotropic extracts had the effect, not of inhibiting, but of augmenting the action of the corresponding extract on test rats. This effect was produced by the serum of a sheep which had received a course of injections with extract of sheep pituitary, and by that of a goat receiving pig pituitary extract. Collip (1937) has shown that the serum of sheep injected with sheep pituitary extract augments the action of the injected extract in test rats. A similar result was obtained by Thompson (1937*a*), who found that the serum of dogs and a horse after injection of extracts of sheep pituitary showed temporary pro-gonadotropic activity. One of the explanations suggested by

Thompson was based on the possibility that the gonadotropic antagonist (Evans and others 1936; Freud 1937) is antigenic, and that the augmenting sera contain a predominance of antibody against the antagonist.

METHODS

Extracts—An extract of acetone-desiccated sheep anterior pituitary was made by three successive extractions with 20 vol. of 70 % alcohol at *p*H 8·5–9. The supernatant fluid, after centrifuging, was acidified to *p*H 5·5, and the extract precipitated with 3 vol. of alcohol. The precipitate when dry (AP 34 B) represented 2 % by weight of original dry gland. Another sheep pituitary extract (AP 36 B) used in testing, was prepared in a similar manner, except that 60 % alcohol was used in the initial extraction. A pig pituitary extract (AP 43 D) and a horse pituitary extract (AP 41 D) were prepared from desiccated glands by four aqueous extractions at *p*H 8·5–9, the extract being precipitated by the addition of 5 vol. of alcohol. The ox pituitary extract (AP 15 B) used has been previously described (Parkes and Rowlands 1936). The extract of human urine of pregnancy (UP 10) was that assayed by Deanesly (1935).

Commercial preparations used included Antex Leo (PMS 3), an extract of pregnant mare serum (Løvens Kemiske Fabrik), and Ambinon (AP 51), a pig pituitary extract (Organon Laboratories).

Animals used in production of serum—An adult castrated ram (sheep 4), was injected subcutaneously daily with 100 mg. (about 1·5 g. dried gland) of the sheep pituitary extract AP 34 B from 10 November 1936 to 25 January 1937; subsequently the daily amount injected was increased to 150 mg. for a further period of 9 weeks to 29 March 1937. Blood samples were taken from the sheep at intervals and after the 19 weeks of injections the animal was bled on 30 March 1937 under anaesthesia from the carotid artery; approximately 750 c.c. of serum were obtained. The serum was Seitz filtered and kept in cold storage.

An adult goat (goat 2) was injected in a similar manner with 100 mg. of pig pituitary extract (Ambinon) for 14 weeks (3 December 1936–15 March 1937), followed by 170 mg. daily of the extract AP 43 D for another month. The animal was then bled under anaesthesia (15 April 1937); 650 c.c. of serum were obtained and stored as that from the sheep.

Tests applied—Two methods were used to observe the action of these sera in modifying the effects of gonadotropic extracts:

1—Groups of rats (40–50 g.) were injected once daily subcutaneously for 5 days, and killed 1 day after the last injection; the ovaries and uterus were fixed in Bouin's fluid and weighed on a torsion balance from 70 % alcohol. The weight of the ovaries of the normal rat at this body weight is about 10 mg. Gonadotropic activity is revealed by growth to a weight in excess of this; antigonadotropic activity by hindrance of this growth promotion. Assay can be effected by determining the minimum dose of serum which is necessary to neutralize the effect of a particular dose of the gonadotropic extract. Except where otherwise stated, the extract and the serum were injected separately on opposite sides of the animal. It was in relation to this test alone that the two sera described in this paper were found to have an augmenting action.

2—Groups of ten oestrous rabbits were injected intravenously and examined next day for the occurrence of ovulation, by the technique of Hill, Parkes and White (1934). It will be seen that neither serum had an augmenting effect on gonadotropic activity thus assessed.

The sera were also tested against the thyrotropic activity of the corresponding extracts by observing the effect on the weight of the thyroid glands in groups of female guinea-pigs of 200 g. body weight, receiving daily injections for 5 days (Rowlands and Parkes 1934).

I—EFFECT OF THE GONADOTROPIC EXTRACTS ALONE

The responses produced by the various gonadotropic extracts alone are shown in Tables I and IV, in the lines where the serum given is entered as "none". From these tables it will be seen that the sheep and pig pituitary extracts are but poorly active even in large doses, while the ox pituitary extract shows only the slightest activity. The horse pituitary extract is, however, highly active.

II—EFFECT OF ADDITION OF NORMAL SERUM

In view of the evidence that normal serum and several non-specific tissue extracts can increase the effectiveness of pituitary extracts (Evans, Simpson and Austin 1935; Cassida 1936; Hellbaum 1936; McShan and Meyer 1937) it was first necessary to find out to what extent any augmentatory effect of normal serum was due to the introduction of a large amount of foreign material affecting the rate of absorption of the extract. Experiments were therefore carried out on the effect of the injection of normal goat or sheep serum on the extracts used. The results are given in Table I. The normal

Table I—The Effect of Serum, Injected in Different Ways, on Sheep and Pig Pituitary Extracts. 1 c.c. of serum was injected once daily for 5 days

Gonadotropic preparation			Serum			Weight of rat organs	
Source	No.	Amount given (mg.)	Nature	Method of injection	No. of rats used	Ovaries (mg.)	Uterus (mg.)
Sheep pituitary	AP 36B	15	None	—	15	23	32
,,	AP 36B	15	Normal sheep	On either side of rat	5	30	34
,,	AP 36B	15	,,	Mixed in syringe	4	37	47
,,	AP 45D	15	None	—	10	36	29
,,	AP 45D	15	Normal goat	On either side of rat	10	47	47
,,	AP 36B	15	None	—	15	23	32
,,	AP 36B	15	Sheep 4 serum	On either side of rat	10	76	60
,,	AP 36B	15	,,	Mixed in syringe	3	53	50
Pig pituitary	AP 43D	25	None	—	5	27	34
,,	AP 43D	25	Normal goat	On either side of rat	10	53	42
,,	AP 43D	25	,,	Mixed in syringe	5	37	39
,,	AP 44	25	None	—	10	32	43
,,	AP 44	25	Normal goat	On either side of rat	5	36	38
,,	AP 51	25	None	—	15	22	25
,,	AP 51	25	Normal goat	On either side of rat	10	27	25
,,	AP 43D	25	Goat 2 serum	,,	10	72	62
,,	AP 43D	25	,,	Mixed in syringe	4	72	94

sheep serum caused little increase in the response evoked by the sheep pituitary extract, but the normal goat serum clearly augmented that due to the pig pituitary extract (AP 43 D), increasing the ovarian response in the rat from 27 to 53 mg. Two other extracts of pig pituitary (AP 44 and 51) were not, however, augmented by the normal goat serum. The sheep pituitary extract AP 45 D was slightly affected by the normal goat serum. The most obvious explanation of these results is that, in spite of the separate injection, there is enough mixing of the extract and the serum in the subcutaneous space to delay the absorption of the hormone, and thus to give the enhanced effect which can be obtained by multiple injections or by addition of a precipitant, such as zinc sulphate, to the solution.

The next experiment, therefore, consisted of mixing the extract and the serum in the syringe before injection, so that the maximum mechanical effect might be exerted. With this modification the augmenting effect of normal sheep serum appeared to be greater than with separate injections, but the anomalous result previously obtained by the separate injection of normal goat serum and pig pituitary extract was not confirmed. Except for this anomalous result, the augmentation produced by normal sera was much less than that given by the sera from the sheep and goat receiving the pituitary extracts, which is the main subject of the investigation here recorded.

III—EFFECT OF ADDITION OF SERA OF ANIMALS WHICH HAD RECEIVED COURSES OF INJECTIONS

a—Tests on the Immature Rat

The sheep serum was first tested for any augmenting effect on the action of sheep pituitary extract after about three months of daily injections; it was then found to augment the action of a saline extract of sheep pituitary (see Table II). The serum by itself had no gonadotropic activity, since neither vaginal opening nor ovarian response occurred in rats injected with it alone (Table II).

The effect of various amounts of this serum on a constant amount of sheep pituitary extract is given in Table III, which shows that a fivefold augmentation of the activity of the extract is obtained in rats by the addition of a total of 5 c.c. of the serum to 15 mg. of the extract.

The prolonged injection of the goat with pig pituitary extract was originally undertaken in the hope of producing anti-thyrotropic serum, but after 3½ months the serum was tested for its effect on the gonadotropic activity of the pig pituitary extract and was found to augment the response (Table IV) to a much greater degree than did normal goat serum.

After showing that the serum of the sheep injected with sheep pituitary, and of the goat injected with pig pituitary, augmented the response of their respective extracts in rats, their effect on the same response, as produced by extracts of pituitaries of other species, as well as by extracts of pregnant mare serum and of human urine of pregnancy, were investigated. The results are shown in Table IV. It can be seen that the serum of the sheep receiving injections of sheep pituitary did not augment the activity of any of the other gonadotropic preparations, nor did it show any inhibitory activity. The serum of the goat injected with pig pituitary extract, however, trebled the effect of the sheep extract, and produced a significant augmentation of the response given by ox pituitary extract which, if given alone, is relatively inactive on the rat ovary. This goat serum not only failed to augment the gonadotropic activity of horse pituitary, pregnant mare serum and an extract of human urine of pregnancy, as tested on the growth of rat ovaries, but it exerted a quite definite inhibitory effect. In other words, towards these extracts it shows the ordinary antigonadotropic activity which is usually evoked by the prolonged injection of gonadotropic extract.

TABLE II—EFFECT OF SERUM FROM SHEEP 4 ON A SALINE EXTRACT OF SHEEP PITUITARY

Amount of extract given (g.)	No. of rats	Amount of serum (c.c.)	Period tested after first injection	Average weight of rat organs	
				Ovaries (mg.)	Uterus (mg.)
1·0	5	None	—	48	48
1·0	5	5	3 months	105	57
1·0	5	5	3½ „	78	71
None	5	5	3½ „	12	22

TABLE III—THE EFFECT OF VARYING AMOUNTS OF SHEEP SERUM ON A CONSTANT AMOUNT (15 mg.) OF SHEEP PITUITARY EXTRACT (AP 36B)

Amount of serum (c.c.)	No. of rats used	Average body weight of rats (g.)	Average weight of rat organs		Increase in weight of ovary over normal (mg.)	No. of times ovarian response is augmented
			Ovaries (mg.)	Uterus (mg.)		
None	15	57	23	32	13	—
Sheep 4 2·5	10	51	31	44	24	1·8
„ 3·75	10	48	40	53	30	2·3
„ 5·0	10	51	76	40	66	5·0
Normal sheep serum 5·0	5	50	30	34	20	1·5

TABLE IV—THE EFFECT OF A CONSTANT AMOUNT (5 c.c.) OF SHEEP 4 AND GOAT 2 SERUM ON VARIOUS GONADOTROPIC PREPARATIONS

Gonadotropic preparation					Weight of rat organs			
					Sheep 4 serum		Goat 2 serum	
Source	No.	Amount given (mg.)	No. of rats used	Amount of serum given (c.c.)	Ovaries (mg.)	Uterus (mg.)	Ovaries (mg.)	Uterus (mg.)
Pig pituitary	AP 43 D	12·5	10	None	—	—	22	26
	AP 43 D	12·5	10	5	—	—	37	39
	AP 43 D	25	5	None	27	24	27	24
	AP 43 D	25	5, 10	5	33	30	72	62
	AP 51	25	15	None	—	—	22	25
	AP 51	25	7	5	—	—	45	34
Sheep pituitary	AP 36 B	15	15	None	23	32	23	32
	AP 36 B	15	10	5	76	60	47	60
Ox pituitary	AP 15 B	50	5	None	15	22	15	22
	AP 15 B	50	5, 10	5	19	26	25	31
Horse pituitary	AP 41 D	0·5	10	None	33	63	33	63
	AP 41 D	0·5	8, 6	5	34	46	17	68
Pregnant mare serum	PMS 3	2·5	15	None	59	97	59	97
	PMS 3	2·5	10	5	57	83	14	28
Human urine of pregnancy	UP 10	0·5	10	None	29	68	29	68
	UP 10	0·5	10	5	29	68	14	19

b—Tests on the Oestrous Rabbit

The results obtained on the effect of these sera on the ovulation-producing capacity of sheep and pig pituitary extracts are shown in Table V.

The serum of the sheep injected with sheep pituitary extract failed to alter, in either direction, the capacity of the sheep pituitary extract (AP 36 B) to cause ovulation in the oestrous rabbit. The serum of the goat injected with pig pituitary extract, however, considerably inhibited the power of this extract to cause ovulation, an effect not given by normal goat serum. It seems significant, therefore, that although these sera augmented the action of sheep and pig pituitary extracts in promoting ovarian growth in the immature rat, no such effect was observed of increasing the ovulation-producing capacity of the same extracts in the oestrous rabbit. Moreover, the goat serum which augmented the response of the pig extract in rats, inhibited the capacity of the same extract to cause ovulation in rabbits.

TABLE V—THE EFFECT OF SERUM FROM SHEEP 4 AND GOAT 2 ON THE OVULATION-PRODUCING CAPACITY OF EXTRACTS OF SHEEP AND PIG PITUITARIES ON OESTROUS RABBITS

Gonadotropic substance		Serum used			
Nature	Amount given (mg.)	Nature	Amount (c.c.)	No. of rabbits used	Percentage ovulation
AP 36B (sheep)	0·15	None	—	10	40
,,	0·15	Sheep 4	4	10	30
AP 43D (pig)	0·1	None	—	10	70
,,	0·1	Goat 2	4	10	10
,,	0·1	Normal goat serum	4	10	70

c—The Effect of Sera on Thyrotropic Activity of Extracts

The extracts of sheep and pig pituitary were respectively slightly and highly thyrotropic to the immature guinea-pig. The serum of the sheep and goat that had received courses of injections of pituitary extracts showed no signs of augmenting this activity. The goat serum, on the contrary, was slightly antithyrotropic.

DISCUSSION

The treatment of a sheep with a course of injections of extract of sheep's anterior pituitary lobe was originally undertaken with the object of testing whether, under such conditions, an antibody to one or another of the anterior pituitary hormones would be formed. All the earlier evidence for the formation of such antibodies had been obtained by injecting animals with extract from the pituitaries of another species, e.g. rats or rabbits with extracts of ox pituitary. If the antibody which appeared under these conditions was truly an immune body, as the evidence seemed to indicate, it was probable that it would not be formed, or that it would, at best, be formed much less readily, when an animal received injections of extracts from pituitaries of its own species. The results are in accordance with this expectation, in that the serum of the above sheep exhibited no obvious neutralizing effect on the action of any of the gonadotropic hormones, or other hormones of the anterior pituitary lobe of any species, so far as these were tested. On the other hand, the serum of the goat which had received a course of injections of pig pituitary extract, inhibited the gonadotropic activity of extract from horse pituitary, from the serum of pregnant mares and from human urine of pregnancy, as exhibited by the power of these extracts to cause growth of the ovaries and uterus of the immature rat. The goat's serum inhibited also the gonadotropic activity of the pig pituitary extract used in producing it, when the test for this activity was made on the oestrous rabbit. It also exhibited some power to inhibit the thyrotropic action of anterior pituitary extracts. So far, then, the results show a sharp contrast between the absence of antihormone activity from the serum of the sheep injected with sheep pituitary, and the presence of such activity in the serum of the goat injected with pig pituitary. The results are in accordance with immunological expectation. They confirm Thompson's (1937*b*) results on sheep injected with sheep pituitary extracts, but differ from those of Collip (1937) who found that, with a long continued course of injections with sheep pituitary, the serum of a sheep, after an augmenting phase, eventually acquired an antigonadotropic action. The evidence hitherto available, from all three sources, shows that, in any case, the production of an antihormonal serum by injecting a sheep with sheep pituitary extract is a matter of much greater difficulty and uncertainty than the production of antisera to pituitary or analogous hormones by giving an animal a course of injections with extracts from an alien species.

Much more difficult to interpret is the acquisition by certain sera of an augmenting rather than an inhibiting effect on a specific, gonadotropic activity of the pituitary extract used for the course of injections. According to Collip, as already mentioned, such an action was observed in the case of the serum of a sheep injected with pituitary extracts from the same species. The serum showing this augmenting action was only obtained during the earlier part of the course of injections and Collip found that later, even with the injection of iso-specific extracts, the augmenting effect is ultimately replaced by an inhibiting effect. It has been shown by Thompson (1937*a*), who injected sheep pituitary extract into dogs and a horse, and in the present work, by the injection of pig pituitary extract into a goat, that augmenting sera can result from allo-specific injections.

There are other features of this augmenting action which need to be considered in attempting to assess its significance. These are as follows:

1—It has been produced by the injection only of anterior pituitary extracts, and not of other gonadotropic extracts.

2—It has been demonstrated only on the gonadotropic activity of the pituitary body and not hitherto on any other pituitary hormonal activity, or on the gonadotropic activity of any other kind of extract.

3—The action is further limited to that particular measure of gonadotropic activity which is obtained by the use of immature female rats. It is the growth of the ovaries and uterus in such animals, under the influence of pituitary injections repeated during several days, which the serum augments. A serum, like that from the goat injected with pig pituitary, here described, may show a normal inhibition of the action of its evocative extract in causing ovulation in the rabbit, and yet augment the promotion, by the same extract, of the growth of the ovaries and the uterus in the immature rat.

It is clear, therefore, that the augmentatory, or pro-gonadotropic action of such sera is on a very different footing from the antigonadotropic action shown by other sera, or even under different conditions by the same sera. There is now good reason for regarding the inhibiting constituent of the antigonadotropic sera as a true antibody in the immunological sense; but there is less reason for regarding the pro-gonadotropic principle as due to an immunity reaction. It must be remembered that a growth response, like that which gives the measure of gonadotropic activity in the rat test, is particularly susceptible to factors influencing the rate of absorption and excretion of the exciting hormone, delay of absorption frequently augmenting the response as thus measured. Some of these experiments suggest a minor augmentation by normal sera of the gonadotropic activity of pituitary extract as thus tested, presumably due to delay of absorption. It would not be difficult to suppose that the long-continued daily injection of pituitary extracts, apart from any formation of inhibitory antibodies which it might evoke, might cause some change in the serum which would intensify such a normal delaying action on absorption.

The fact that the augmenting activity appears only when the serum is given with sheep, pig and ox pituitary extracts and that the augmentation is much greater than can be obtained with normal goat or sheep serum, suggests that the effect is specific. If the specificity of the augmentatory effect is substantiated, it is likely that the fact that one and the same sample of serum may be antigonadotropic to one type of extract and pro-gonadotropic to another, will find its explanation in connexion with the pituitary antagonist described by Evans and others (1936). If crude extracts of sheep and pig pituitary contain gonadotropic hormone and antagonistic substance, and both are antigenic, then the antiserum may contain antibodies to both. If the antagonist is the better antigen, the antiserum may first become more potent in anti-antagonistic than in antigonadotropic action, and may thus increase the effectiveness of extracts containing much antagonist by neutralizing the latter to a greater extent than it neutralizes the gonadotropic hormone. On the other hand, gonadotropic preparations containing little or no antagonist (horse pituitary, pregnant mare serum and urine of pregnancy) would be more affected by the antigonadotropic power of such a serum, and would consequently be inhibited. The observation of Collip (1937) and Thompson (1937*a*) that the production of pro-gonadotropic serum is only temporary could be explained similarly, if the antigonadotropic substance in the serum were to increase as injections were continued, so that even if the serum neutralized the antagonistic factor, the neutralization of the gonadotropic substance would become predominant and inactivate the extract.

SUMMARY

The serum of a sheep receiving a course of injections of extract of sheep pituitary did not show antigonadotropic activity, but possessed the power to augment the activity on test rats of an extract prepared from the same species.

The serum of a goat injected with pig pituitary extract augmented the action of pig, sheep and ox pituitary extracts in promoting ovarian growth in the immature rat; at the same time, this serum inhibited the ovulation-producing activity of the same extract of pig pituitary in the oestrous rabbit. The serum also inhibited the gonadotropic action on the rat ovary of extracts of horse pituitary, pregnant mare serum and human urine of pregnancy.

This augmentatory action is perhaps explicable by reference to Evans's pituitary antagonist.

REFERENCES

Cassida, L. E. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **33**, 570–2.
Collip, J. B. 1937 *Canad. Med. Ass. J.* **36**, 199–200.
Deanesly, R. 1935 *Quart. J. Pharm.* **8**, 651–68.
Evans, H. M., Simpson, M. E. and Austin, P. R. 1935 *J. Exp. Med.* **58**, 561–8.
Evans, H. M., Korpi, K., Pencharz, R. I. and Simpson, M. E. 1936 *Univ. Calif. Publ. Anat.* **1**, 237–54.
Freud, J. 1937 *Nature, Lond.*, **139**, 880–1.
Hellbaum, A. A. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **33**, 568–70.
Hill, R. T., Parkes, A. S. and White, W. E. 1934 *J. Physiol.* **81**, 335–60.
McShan, W. H. and Meyer, R. K. 1937 *Amer. J. Physiol.* **119**, 574–9.
Parkes, A. S. and Rowlands, I. W. 1936 *J. Physiol.* **88**, 305–11.
Rowlands, I. W. 1937 *Proc. Roy. Soc.* B. **124**, 503–521.
Rowlands, I. W. and Parkes, A. S. 1934 *Biochem. J.* **28**, 1829–43.
Thompson, K. W. 1937*a* *Proc. Soc. Exp. Biol., N.Y.*, **35**, 640–4.
— 1937*b* *Proc. Soc. Exp. Biol., N.Y.*, **35**, 634–7.

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The Specificity of Antigonadotropic Sera

By I. W. ROWLANDS

From the National Institute for Medical Research, Hampstead, N.W. 3

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The original work of Collip and his colleagues on "antihormones" has been confirmed in its main features by many workers, and it is now clear that the prolonged injection of an extract containing a gonadotropic hormone into an animal of a different species leads to the recipient becoming insensitive to its effects. At the same time, the serum of the recipient acquires the power to neutralize the antigenic extract in test animals. Since gonadotropic extracts can be prepared from all animal pituitaries so far examined, and from the blood serum of pregnant mares and women, as well as from human placenta and urine of pregnancy, a wide field of specificity investigation is opened up.

Several groups of workers have reported on the specificity of antigonadotropic substances, produced under varying conditions and in different species of animals. Collip and his co-workers (Collip 1934) showed that rats rendered refractory to gonadotropic extracts of non-human pituitaries may be sensitive to extracts of human urine of pregnancy. Fluhmann

(1935*a*) produced in the rat an antiserum to human pituitary extract, which was found to neutralize not only the effect of the antigenic extract against which it was prepared, but also that of an extract of human pregnancy serum. It did not, on the other hand, prevent the action of sheep pituitary extract administered to immature rats. In a later paper, Fluhmann (1935*b*) records the preparation of an antiserum to an extract of human pregnancy serum, which did not inhibit human or sheep pituitary extract.

An antiserum to a gonadotropic extract of pregnant mare serum was prepared in monkeys by Gustus, Meyer and Dingle (1935), which, although inhibitory to the extract against which it was prepared, would not significantly neutralize extracts of human pregnancy serum or of sheep or human pituitary. Thompson and Cushing (1937) found that the antiserum to extract of pregnant mare serum, prepared in a dog, would only partially inhibit the gonadotropic activity of an extract of sheep or pig pituitary, or of human urine of pregnancy. The serum of two dogs immunized with extract of sheep pituitary was less specific, and neutralized extracts of pig pituitary, human urine of pregnancy, human urine of menopause and pregnant mare serum.

Twombly (1936) has shown that antiserum raised in rabbits, to a gonadotropic extract of urine of patients with teratoma testis, inhibited the activity of human urine of pregnancy extract. Brandt and Goldhammer (1936), also, found that antiserum to human urine of pregnancy extract will neutralize gonadotropic extracts from the other naturally occurring sources within the human body, viz. placenta, pituitary and pregnancy serum. This antiserum, however, would not inhibit a gonadotropic extract prepared from the serum of pregnant mares. On the other hand, Gegerson, Clark and Kurzrok (1936) found that an antiserum made by injecting an extract of human urine of pregnancy neutralized the ovulation-producing activity of ox pituitary extract, but that an antiserum made by injecting the latter did not inhibit the urine of pregnancy extract. Both these antisera, however, inhibited extract of pregnant mare serum.

Zondek and Sulman (1937) have recently studied the specificity of antigonadotropic sera. Their experiments, based on antisera to a gonadotropic extract from human urine of pregnancy and to one from pregnant mare serum, lead them to conclude that there is a strict species-specificity and also a relatively high organ specificity.

The present paper records an investigation into the specificity of antigonadotropic sera, made as a sequel to the early experiments described by Rowlands (1937*a*). Some of the features of this work have been reported recently in abstract (Rowlands 1937*b*).

MATERIAL

Extracts used to produce antisera—Four different gonadotropic preparations were used for the production of antigonadotropic sera, viz. urine of pregnancy extract ("Pregnyl"), pregnant mare serum extract ("Antex"), ox pituitary extract and horse pituitary extract.

The method of preparation of the ox pituitary extract by 50 % pyridine extraction (Fevold, Hisaw and Leonard 1931) has been previously described (Rowlands 1936). The horse pituitary extract was prepared in a similar manner. In both cases the extract was of such strength that 1 part of extract represented 50 parts of the corresponding fresh anterior pituitary substance.

The production of antisera—The data relating to the production of the antisera are shown in Table I. Injections were made subcutaneously once daily; the animals were bled once weekly after it had been shown that the serum was active. From each rabbit about 20 c.c. of blood was withdrawn from the marginal ear vein each week and from the goat about 200 c.c. Larger batches of serum were obtained when the animals were bled out at the end of the experiment.

TABLE I—THE METHOD OF PRODUCTION OF ANTIGONADOTROPIC SERA

Species injected	Gonadotropic extract of	Amount injected daily (mg.)	Period of injections (months)
Rabbit	Ox pituitary (AP 22 B)	25·0	9
,,	Horse pituitary (AP 25 B)	10·0	5½
,,	Pregnant mare serum (PMS 2)	0·5–3·0	4½
,,	Urine of pregnancy (UP 11)	0·5	8½
Goat	Urine of pregnancy (UP 12)	10·0–20·0	5

Assay—The most convenient assay method for this work is that based on weight changes in the ovary of the immature rat. Groups of five or ten female rats weighing 40–50 g. were injected once daily for 5 days and killed 24 hr. afterwards. The ovaries were decapsulated, fixed in Bouin's fluid and weighed on a torsion balance from 70 % alcohol. Without treatment the ovaries weigh 10–12 mg. The horse pituitary extract, in a total dose of 5 mg., produced ovaries weighing about 66 mg. The urine of pregnancy extracts (UP 11 and UP 12) gave a nearly maximum response in the rat ovary with 0·25 mg., while a total dose of 2·5 mg. of the pregnant mare serum extract used (PMS 2) produced ovaries weighing 29 mg.

For many combinations of extracts and antisera tests were carried out on both immature rats and oestrous rabbits (see Table III). Specific reference to the rabbit experiments is made only when the results differ from those on the rat, or when, as with ox pituitary extracts, rat tests were impracticable. The test depending on ovulation in the oestrous rabbit consisted of a single intravenous injection of the extracts followed by a laparotomy 24 hr. later. The amount of ox pituitary extract necessary to produce ovulation in 50 % of a group of ten rabbits was about 1 mg.

Other extracts used—Several other gonadotropic extracts were used in this work. Saline extracts of fresh sheep and pig anterior pituitaries were made by mincing the glands and extracting twice with vigorously stirred saline for 2 hr. Extract equivalent to 1 g. of the fresh sheep pituitary injected over 5 days to a group of five rats produced ovaries weighing 48 mg. Similar treatment with the same amount of pig pituitary yielded ovaries weighing 43 mg. The other sheep and pig pituitary extracts used in testing were made from acetone desiccated glands by three extractions with 70 % alcohol at *p*H 8, acidification of the extract to *p*H 5·5 and precipitation of the active material with three volumes of ethyl alcohol.

Several gonadotropic preparations of human origin were used. Assay of each of these substances was performed on the immature rat. Two acetone desiccated human pituitaries (HP3 and 4) from patients of 60–65 years were used, and extract equivalent to 3 mg. of the dry powder produced an ovarian weight of 59 mg. A saline extract of fresh human pituitaries was also used, of which 25 mg. evoked ovaries weighing 69 mg. A saline extract of human placenta was made of which the equivalent of 10 g. original tissue over a 5-day period produced ovaries of an average weight of 67 mg. A similar extract of another placenta showed almost identical activity. A mixed batch of human pregnancy serum (4–6 months) was also obtained; a total of 1·5 c.c. given over a period of 5 days produced an average ovary weight of 34 mg.

RESULTS

The efficiency of the antisera in inhibiting the effects of the gonadotropic extracts against which they were prepared—It was shown, in the first place, that each antiserum was capable of neutralizing the effect on test animals of the gonadotropic extract against which it was prepared. The minimal amount necessary to effect complete inhibition is shown in Table II.

It is interesting to compare the activities of the two antisera prepared with extracts of human urine of pregnancy. The total amount of "Pregnyl" injected into the goat was nearly double, in proportion to the body weights, that received by each rabbit over a period of 5 months. The total activity of antiserum obtained from each can be represented by the equivalent amount of gonadotropic material. For the rabbit, the antiserum was equivalent to about half the gonadotropic material administered, while the goat antiserum was equivalent to about ten times the amount given. The goat antiserum after 3 months' injection (Rowlands and Parkes 1937) was equivalent to about twenty times the amount injected.

TABLE II—THE ACTIVITY OF THE ANTIGONADOTROPIC SERA, TESTED IN RATS, AGAINST THE IMMUNIZING EXTRACTS

Antiserum prepared against	Species prepared in	Amount of extract given to test rats mg.	Weight of ovaries of test rats mg.	Minimal amount of serum for complete inhibition c.c.
UP 11	Rabbit	1	34	0·25
UP 12	Goat	1	34	0·0125
Horse pituitary	Rabbit	5	70	0·5
Pregnant mare serum	,,	2·5	30	0·5

TWO TYPES OF SPECIFICITY

From the records of the earlier investigations mentioned above, it is seldom possible to draw a clear conclusion as to which of two possible types of specificity might be involved in a particular differential effect. When, for example, it is shown that an animal, rendered immune to an extract from anterior pituitary of non-human origin, still reacts to an extract from human urine of pregnancy, we have no data for a decision whether the immunity discriminates between species, or between the pituitary and the urinary hormone. No test made of the neutralizing power of a serum, on the gonadotropic action of an extract made from an organ (or fluid) of a species *both* different from those which yielded the immunizing extract, will provide evidence for such a decision. In these experiments each of the five antisera available has been tested for its power of inhibiting the gonadotropic action of as many different extracts as possible, made from different organs and fluids of different species. The object has been to determine, in each case, the degree to which the neutralizing action shows two kinds of specificity: (1) "species-specificity"—the degree to which the neutralizing action is limited to *the particular species* which yielded the immunizing extract, or extends to the corresponding organ or fluid of other species; and (2) "source-specificity"—the degree to which it is limited to *the particular organ or fluid* which yielded the extract, or extends to gonadotropic extracts from other organs or fluids of the same as well as of other species. Table III shows the combined results.

The detailed results for each serum, as regards its power of neutralizing the gonadotropic activities of a series of extracts as shown by two different biological reactions, are given in Tables V–XII. It will be evident that with a possibility of two types of specificity, either of which might be perfect, partial to any degree, or completely absent, and which might vary in the two tests, the interpretation of some of the results may be difficult. Only if perfect specificity of one or of both types should be present will the results be clear.

Serum 5—A glance at Table III will show that in the case of serum No. 5, obtained by immunizing rabbits against the gonadotropic hormone extracted from pregnant mare's serum, the specificity of both types is perfect, so far as the tests can show. The antibody is "species-specific" for the horse, in that it neutralizes the effect of the hormone from pregnant mare's serum, but completely fails to neutralize that from human serum, the only other pregnancy serum available for test. This serum is also "source-specific", in that it neutralizes the gonadotropic hormone from mare's serum, but has no effect on that from the horse's pituitary body, which was the only other available source of gonadotropic hormone in that species. In this one instance, therefore, both types of specificity, as shown by the two biological reactions, are complete.

Sera 1 *and* 2—These sera, prepared by immunizing rabbits and a goat against the gonadotropic substance extracted from human urine of pregnancy, appear to show a complete "species-specificity". In each case the serum was found to neutralize any gonadotropic hormone of human origin against which it was tested, including the extracts from the pituitary body (fig. 1), from pregnancy serum and from placenta, as well as that from the urine used for immunization. On the other hand, neither showed any inhibitory effect on any gonadotropic hormone from other species with which it was tested. The evidence would be more complete if the sera could be tested against urinary gonadotropic hormones from other species; but hitherto none such has been discovered. So far as it goes, however, the evidence is for the complete "species-specificity" of these sera, and their equally total lack of "source-specificity".

Serum 3—The serum produced by immunizing rabbits against extract of horse pituitary gives reactions which are less easy to interpret. It will be seen that, in addition to horse pituitary extract, it is fully effective only

TABLE III

Antiserum to	Human				Horse		Sheep	Pig	Ox
	Pituitary	Pregnancy serum	Pregnancy urine	Placenta	Pituitary	Pregnancy serum	Pituitary	Pituitary	Pituitary
1. Human pregnancy urine extract UP 11 (rabbit)	Rt + Rb	+ Rb	Rt □ Rb	Rt +	Rt –	Rt –			
2. Human pregnancy urine extract UP 12 (goat)	Rt + Rb	Rt +	Rt □		Rt –	Rt –	Rt –	Rt –	– Rb
3. Horse pituitary extract AP 25B (rabbit)	Rt ⊕	Rt ⊖	Rt ⊖ Rb	Rt ⊕	Rt □	Rt +	Rt –	Rt –	– Rb
4. Ox pituitary extract AP 22B (rabbit)	Rt ⊕ Rb	Rt +	Rt ⊖ Rb	Rt ⊕	Rt + Rb	Rt +	Rt –	Rt –	□ Rb
5. Pregnant mare serum extract PMS 2 (rabbit)	Rt – Rb	Rt –	Rt – Rb	Rt –	Rt – Rb	Rt □ Rb	Rt –	Rt –	– Rb

Gonadotropic substances

The specificity of antigonadotropic sera. *Serum* 1 from rabbits immunized with gonadotropic extract from human urine of pregnancy (UP 11); *Serum* 2 from a goat immunized with a similar extract (UP 12); *Serum* 3 from rabbits immunized with a horse pituitary extract (AP 25B); *Serum* 4 from rabbits immunized with an ox pituitary extract (AP 22B); and *Serum* 5 from rabbits immunized with a gonadotropic extract of pregnant mare serum (PMS 2).

□ = strong neutralization of homologous extract by serum.
+ = strong neutralization of other extracts by serum.
⊕ = weak neutralization.
⊖ = very weak neutralization.
– = no neutralization.

The two biological reactions are indicated as Rt = immature rat test, and Rb = oestrous rabbit test.

against the gonadotropic activity of an extract from mare's pregnancy serum (fig. 2). To that extent it is "species-specific", and this specificity is upheld by its failure to affect the gonadotropic activities of extracts from other ungulate pituitaries (see Tables V and X). The extract of pig pituitary presents a special case, in that its gonadotropic activity, as shown on the immature rat, is apparently enhanced by this serum, as by the anti-pig pituitary serum produced in a goat (see Rowlands 1937*c*). On the other hand, the serum shows a weak neutralizing action on the effects of an

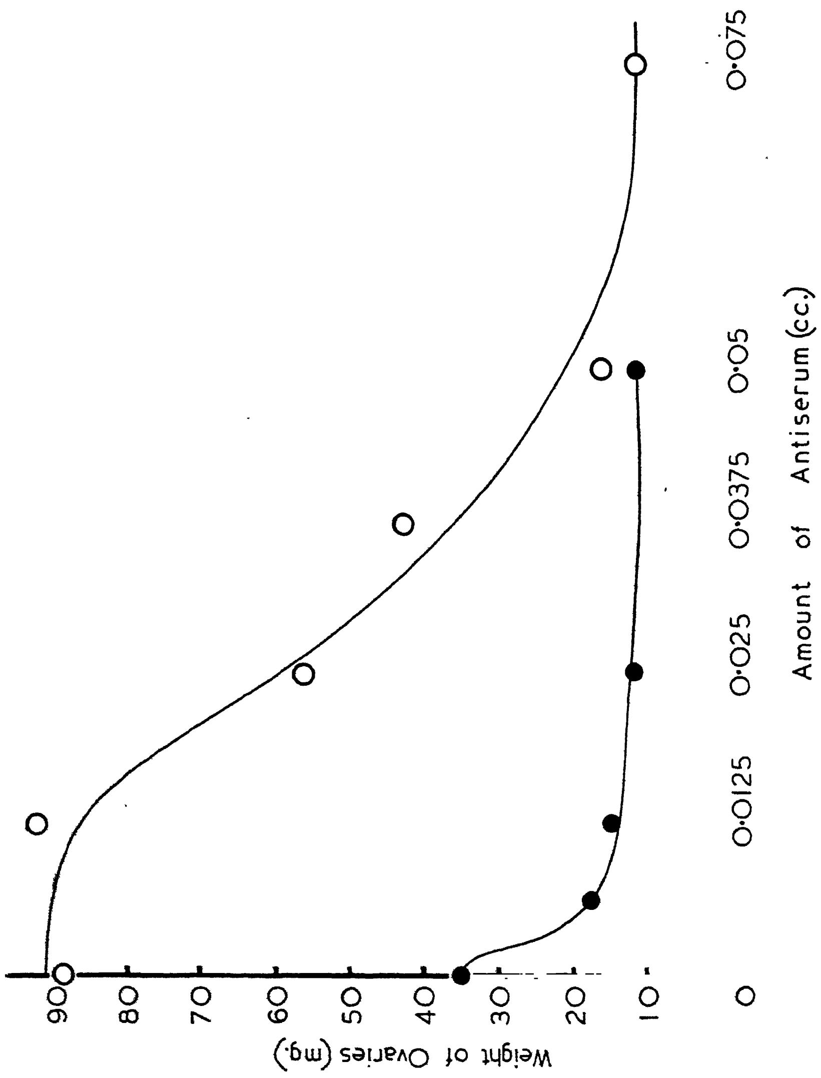

FIG. 1—Comparison of the effectiveness of the antiserum to urine of pregnancy extract in inhibiting urine of pregnancy extract (●) and human pituitary extract (○).

extract from human pituitary (fig. 3), and of one from placenta, and a still weaker action of the same kind on the effects of extracts of human pregnancy urine and human pregnancy serum.

The weak neutralizing action on human pituitary extract might, by itself, suggest a vitiation of the "species-specificity" of the serum by a "source-specificity"; but this suggestion would not explain the action on the gonadotropic extract from extra-pituitary human sources. The facts

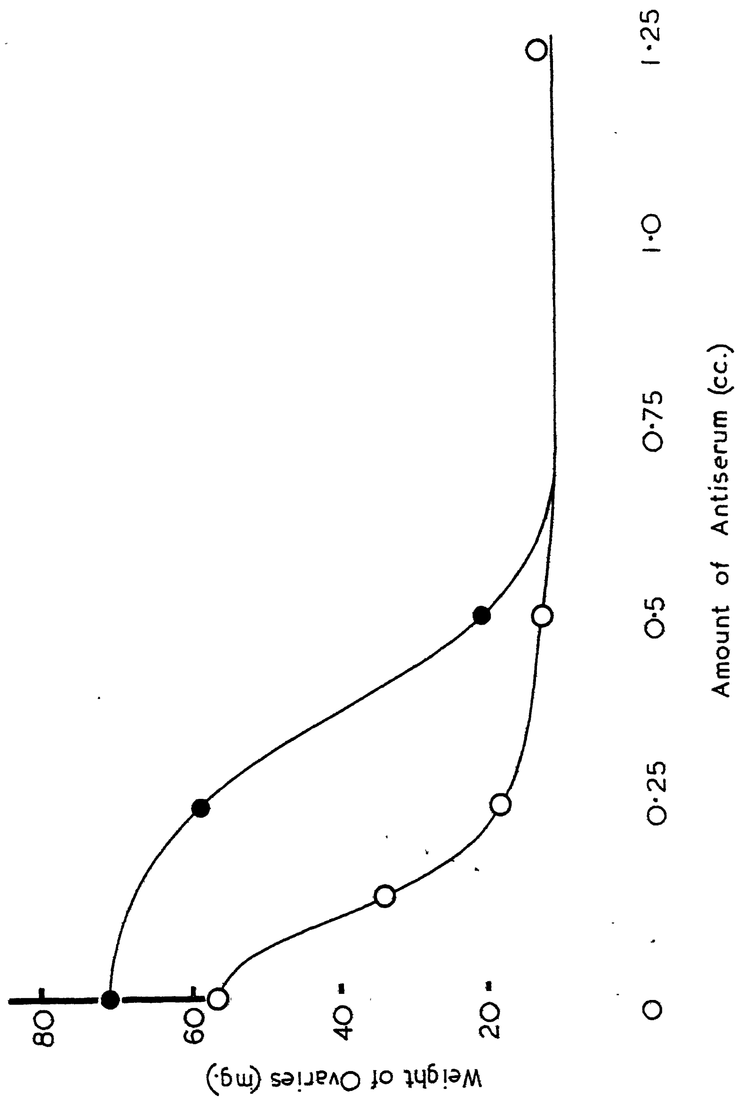

Fig. 2—The comparative effectiveness of antiserum to horse pituitary extract against horse pituitary (●) and pregnant mare serum extract (○).

as a whole suggest for the activity of this serum an incomplete "species-specificity" and no "source-specificity".

Serum 4—A greater breakdown of specificity is seen in the case of the antiserum produced by immunizing rabbits against extracts of ox pituitary. A suggestion of "species-specificity" is to be seen in that, although this serum strongly neutralizes the gonadotropic activity of extract from horse pituitaries, it has only a weak effect on that of a human pituitary extract

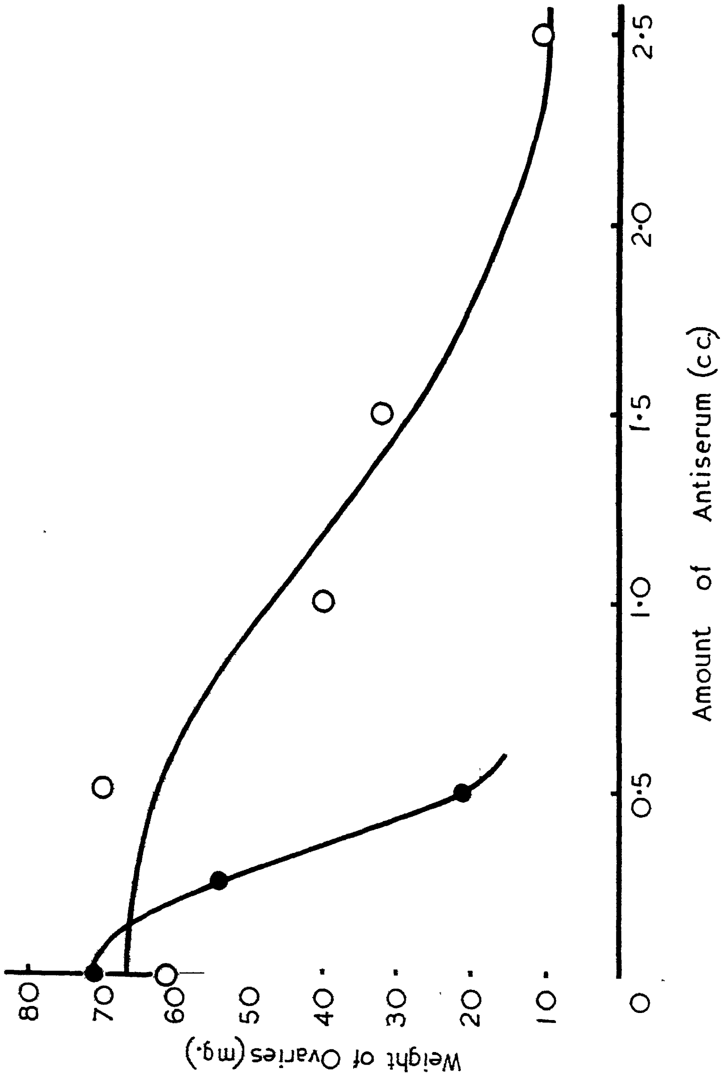

FIG. 3—Comparative effectiveness of an antiserum to horse pituitary against horse pituitary extract (●) and human pituitary extract (○).

and no effect on that of sheep pituitary. In the case of pig pituitary extract no neutralizing action was detected, but, as with Serum 3, an apparent augmentatory action in the test on immature rats. On the other hand, the strong neutralizing action of this serum on gonadotropic extracts from pregnancy serum (both equine and human), and its weaker action on human placenta and urinary extracts, suggest that "source-specificity" is, in this case, as in that of the anti-horse-pituitary serum, completely absent.

The interpretations of the results may conveniently be given in tabular form:

TABLE IV—TYPES OF SPECIFICITY EXHIBITED BY THE ANTISERA

Anti-serum	Species-specificity	Source-specificity
1	Complete	Absent
2	"	"
3	Incomplete (strong)	"
4	Incomplete (very weak)	"
5	Complete	Complete

DISCUSSION

An attempt has been made above to interpret the significance of the results recorded in this paper, taken by themselves. A rather pronounced feature of the results is the paucity of evidence which they afford for "source-specificity", as distinguished from "species-specificity", in the senses defined. This conclusion might, however, be completely changed by more evidence concerning the reactions of some of the sera. Sera 1 and 2, for example, which were prepared by immunizing animals with material from human pregnancy urine, had no effect on any of the gonadotropic preparations from other species which were tested, but these did not include a preparation from urine or placenta. If an active extract of this kind from any other species were available for test, the results might alter the interpretation. Again, though it is shown here that Serum 3, prepared by immunizing with an extract of horse pituitary, does not neutralize the gonadotropic action of a pig pituitary extract, it has been shown elsewhere that an anti-pig pituitary extract does effectively neutralize this action of horse pituitary extract. It has also been shown that Serum 4, produced by immunizing a rabbit against ox-pituitary extract, neutralizes the gonadotropic effect on the rabbit of pituitary extract from other species, and of the pituitary hormone of the rabbit itself, secreted in response to the stimulus of mating (Parkes and Rowlands 1936). The failure to neutralize pig pituitary extract is anomalous and due to a complication earlier discussed. The results with Serum 4 show, further, that such a serum will neutralize not only other pituitary extracts, but also the effects of other gonadotropic preparations from alien species. We seem here, indeed, to reach a condition in which both "species-specificity" and "source-specificity" have broken down, in favour of what may be regarded as a more fundamental "hormone-specificity".

Two other factors must be borne in mind in the interpretation of these results. Firstly, it is now probable that at least two hormones, follicle-stimulating hormone (F.S.H.) and luteinizing hormone (L.H.), are concerned in producing the characteristic gonadotropic effects of the typical pituitary extract. It seems equally certain that the balance of the two hormones is different in preparations from different species. Nothing is known as to the comparative immunological properties of the two substances, and what appears to be species specificity in the effectiveness of antisera might conceivably be due to the fact that the balance of anti-F.S.H. and anti-L.H. present in an antiserum is unsuitable to neutralize the particular combination of F.S.H. and L.H. in the extract of pituitaries of a different species. The only certain fact seems to be that the antibody evoked by the luteinizing substance, as present in human urine of pregnancy, is unable to neutralize the luteinizing substance of animal pituitary, upon which the ovarian-weight response in the immature rat largely depends.

Secondly, there seems to be further scope for complexity of interpretation, arising from the conception of pituitary gonadotropic antagonists. The tables in the present paper show two examples of sera which are antigonadotropic towards the majority of gonadotropic extracts, but have precisely the opposite effect, i.e. an augmentatory action, towards extract of pig pituitary. These are further examples of the phenomena discussed previously (Rowlands 1937c).

It must be kept in mind that there is, hitherto, no evidence for an immune reaction by an animal to any of its own hormones, as naturally secreted, and further that the antibodies here considered are, in all cases except one (Collip 1937), produced by the injection of extracts from organs and fluids in which the hormone is certainly associated with complex and probably antigenic substances specifically foreign to the recipient in almost all cases. It would not be impossible to make a plausible hypothesis as to the meaning of the available data by considering each such extract as a complex antigen, consisting of a species-specific, and in some cases, organ-specific, non-hormonal vector, and an interspecific hormone, not antigenic in itself, but readily functioning as a specific haptene in combination with a vector, and thereby imposing a different and wider specificity. At the present stage, however, the elaboration of such a hypothesis in detail would be of very doubtful value, in view of other factors of uncertainty which still remain.

My best thanks are due to Sir Henry Dale, F.R.S., and to Dr A. S. Parkes, F.R.S., for their assistance and criticism in this and the previous paper, to Dr F. G. Young for several of the pituitary extracts, to Dr M. Boycott for the human pregnancy serum, and to Dr Dorothy Russell for the human pituitaries. I am also indebted to Organon Laboratories and to Løvens Kemiske Fabrik, Copenhagen, for generous supplies of "Pregnyl" and "Antex" respectively.

TABLE V—THE EFFECT OF ANTISERUM TO OX PITUITARY ON GONADOTROPIC EXTRACTS IN THE IMMATURE RAT

Gonadotropic preparation			Amount of anti-serum	No. of rats	Weight of organ	
Source	No.	Amount given	c.c.	used	Ovaries mg.	Uterus mg.
Horse pituitary	AP 2B	10·0 mg.	—	10	74	69
		10·0	0·5	5	21	92
		10·0	2·5	10	10	99
Pregnant mare serum	PMS 3	2·5	—	15	59	97
		2·5	1·25	5	16	23
Sheep pituitary	AP 36B	30·0	—	5	29	28
		30·0	2·5	5	31	33
	Saline extract	1·0 g.	—	5	48	48
		1·0	1·25	5	42	46
Pig pituitary	AP 43D	25·0 mg.	—	5	27	24
		25·0	2·5	5	68	46
	Saline extract	1·0 g.	—	9	43	31
		1·0	1·25	5	79	68
Human pituitary	HP 3, 4	3·0 mg.	—	10	59	55
		3·0	1·25	5	23	106
	Saline extract	25·0	—	5	69	61
		20·0	0·5	5	60	81
Human pregnancy serum	—	1·5 c.c.	—	10	33	59
		1·5	1·25	5	11	24
Human urine of pregnancy	UP 10	1·0 mg.	—	20	34	80
		1·0	0·5	5	29	85
		1·0	1·25	5	22	137
		1·0	2·5	5	17	127
Human placenta	—	10·0 g.	—	5	67	107
		10·0	1·25	5	28	78

Certain supplementary results, especially where these are indicated in the figures, are omitted from these tables.

TABLE VI—THE EFFECT OF ANTISERUM TO HORSE PITUITARY ON GONADOTROPIC EXTRACTS IN THE IMMATURE RAT

Gonadotropic preparation			Amount of anti-serum c.c.	No. of rats used	Weight of organ	
Source	No.	Amount given			Ovaries mg.	Uterus mg.
Horse pituitary	AP33B	5·0 mg.	—	10	71	89
		5·0	0·25	5	54	53
		5·0	0·5	5	21	61
Pregnant mare serum	PMS3	2·5	—	15	59	97
		2·5	0·25	5	19	55
		2·5	1·25	5	12	20
Sheep pituitary	Saline extract	1·0 g.	—	5	48	48
		1·0	1·25	5	46	54
	AP36B	30·0 mg.	—	5	29	28
		30·0	2·5	5	33	34
Pig pituitary	Saline extract	1·0 g.	—	9	43	31
		1·0	1·25	5	53	63
	AP43D	25·0 mg.	—	5	27	24
		25·0	2·5	5	62	56
Human pituitary	Saline extract	25·0	—	5	69	61
		20·0	0·5	5	64	53
	HP3, 4 (acetone desiccated)	3·0	—	10	59	55
		3·0	0·5	5	70	87
		3·0	1·0	5	40	100
		3·0	1·5	5	32	111
		3·0	2·5	5	10	24
Human pregnancy serum	—	1·5 c.c.	—	10	33	59
		1·5	1·25	5	24	82
		1·5	2·5	5	22	100
Human urine of pregnancy	UP10	1·0 mg.	—	20	34	80
		1·0	0·5	5	37	67
		1·0	1·25	5	29	67
		1·0	2·5	5	11	39
Human placenta	Saline extract	10·0 g.	—	5	62	109
		10·0	0·5	5	50	113
		10·0	—	5	67	107
		10·0	1·25	5	31	79

TABLE VII—THE EFFECT OF ANTI-"ANTEX" SERUM ON GONADOTROPIC EXTRACTS IN THE IMMATURE RAT

Gonadotropic preparation			Amount of anti-serum c.c.	No. of rats used	Weight of organ	
Source	No.	Amount given			Ovaries mg.	Uterus mg.
Horse pituitary	AP 33 B	5·0 mg.	—	15	72	88
		5·0	0·5	5	62	68
		5·0	1·25	5	77	80
Pregnant mare serum extract	PMS 2	2·5	—	5	29	54
		2·5	0·5	10	10	24
		2·5	1·25	5	10	17
Sheep pituitary	Saline extract	1·0 g.	—	5	48	48
		1·0	1·25	5	44	42
	AP 36 B	30·0 mg.	—	5	29	28
		30·0	2·5	5	25	30
Pig pituitary	Saline extract	1·0 g.	—	9	43	31
		1·0	1·25	5	50	54
	AP 43 D	25·0 mg.	—	5	27	24
		25·0	2·5	5	43	33
Human pituitary	Saline extract	25·0	—	5	69	61
		20·0	0·5	5	60	63
	HP 3, 4	3·0	—	10	59	55
		3·0	2·5	5	74	64
Human pregnancy serum	—	1·5 c.c.	—	10	33	59
		1·5	1·25	5	32	80
Human urine of pregnancy	UP 10	1·0 mg.	—	20	34	80
		1·0	0·5	5	35	66
		1·0	1·25	5	41	64
Human placenta	Saline extract	10·0 g.	—	5	67	107
		10·0	1·25	5	68	108

TABLE VIII—THE EFFECT OF ANTI-"PREGNYL" SERUM PRODUCED IN A GOAT ON GONADOTROPIC EXTRACTS IN THE IMMATURE RAT

Gonadotropic preparation			Amount of anti-serum c.c.	No. of rats used	Weight of organ	
Source	No.	Amount given			Ovaries mg.	Uterus mg.
Horse pituitary	AP 33B	5·0 mg.	—	15	72	88
		5·0	0·5	5	72	68
Pregnant mare serum	PMS 2	2·5	—	5	29	54
		2·5	0·5	5	31	68
Sheep pituitary	Saline extract	1·0 g.	—	5	48	48
		1·0	1·25	5	46	58
	AP 36B	30·0 mg.	—	5	29	28
		30·0	1·0	5	43	45
Pig pituitary	Saline extract	1·0 g.	—	9	43	31
		1·0	0·5	5 (3 died)	42	65
	AP 43D	25·0 mg.	—	5	27	24
		25·0	1·0	5	29	30
Human pituitary	Saline extract	25·0	—	5	69	61
		20·0	0·5	5	10	17
	HP 5, 7, 8*	3·0	—	10	89	61
		3·0	0·025	10	56	56
		3·0	0·05	10	15	46
		3·0	0·075	10	10	26
Human pregnancy serum	—	1·5 c.c.	—	10	33	59
		1·5	0·5	5	9	17
Human urine of pregnancy	UP 10	1·0 mg.	—	20	34	80
		1·0	0·00625	10	17	74
		1·0	0·0125	10	14	74
		1·0	0·025	10	11	19

* Data taken from Rowlands and Parkes (1937).

TABLE IX—THE EFFECT OF ANTI-"PREGNYL" SERUM PRODUCED IN RABBITS ON GONADOTROPIC EXTRACTS IN THE IMMATURE RAT

Gonadotropic preparation			Amount of anti-serum c.c.	No. of rats used	Weight of organ	
Source	No.	Amount given			Ovaries mg.	Uterus mg.
Horse pituitary	AP 2 B	10·0 mg.	—	10	74	69
		10·0	1·25	5	76	107
Pregnant mare serum	PMS 2	2·5	—	5	29	54
		2·5	0·5	5	23	85
	PMS 3	2·5	—	15	59	97
		2·5	1·25	5	54	73
Human pituitary	Saline extract	25·0	—	5	69	61
		20·0	0·5	5	10	25
Human urine of pregnancy	UP 10	1·0	—	20	34	80
		1·0	0·1	10	24	53
		1·0	0·25	10	10	32
Human placenta	Saline extract	10·0 g.	—	5	62	109
		10·0	0·5	5	18	59

TABLE X—THE EFFECT OF ANTISERUM TO OX PITUITARY ON GONADOTROPIC EXTRACTS IN OESTROUS RABBITS

Gonadotropic preparation			Amount of anti-serum c.c.	No. of rabbits	Percentage ovulation
Source	No.	Amount given			
Horse pituitary	AP 2 B	1·0 mg.	—	10	40
		4·0	0·5	10	10
Human pituitary	Saline extract	10·0	—	10	100
		10·0	1·0	5	0
Human urine of pregnancy	UP 10	0·2	—	10	50
		0·4	0·5	10	80
		0·4	1·0	10	10
Ox pituitary	AP 15 B	0·75*	—	—	50
		4·0	0·5	10	70
		3·0	0·5	10	20
		6·0	1·0	10	20

* Data taken from Parkes and Rowlands (1936).

TABLE XI—THE EFFECT OF ANTISERUM TO HORSE PITUITARY ON GONADOTROPIC EXTRACTS IN OESTROUS RABBITS

Gonadotropic preparation			Amount of anti-serum c.c.	No. of rabbits	Percentage ovulation
Source	No.	Amount given			
Ox pituitary	AP 15B	4·0 mg.*	—	10	100
		4·0	4	10	90
Human urine of pregnancy	UP 10	0·4	—	10	100
		0·4	4	10	90

* Data taken from Parkes and Rowlands (1936).

TABLE XII—THE EFFECT OF ANTI-"PREGNYL" SERUM PRODUCED IN THE RABBIT (R) AND GOAT (G) ON GONADOTROPIC EXTRACTS IN OESTROUS RABBITS

Gonadotropic preparation			Amount of anti-serum c.c.	No. of rabbits	Per-centage ovulation
Source	No.	Amount given			
Ox pituitary	AP 15B	4·0 mg.	—	10	100
		4·0	2 (G)	10	100
Human pituitary	Saline extract	10·0	—	10	100
		10·0	2 (G)	10	0
Human pregnancy serum	—	0·25	—	3	100
		0·5	1 (R)	7	15
Human urine of pregnancy	UP 10	0·5	—	10	100
		0·5	0·5 (R)	4	0

SUMMARY

The specificity of various antigonadotropic sera has been investigated, and an attempt has been made to classify the reactions according to "species-specificity" and "source-specificity".

Antisera were obtained to gonadotropic extracts of ox pituitary, horse pituitary, human urine of pregnancy and pregnant mare serum. All were prepared in rabbits, except an additional antiserum to urine of pregnancy extract which was prepared in a goat. The activity of the antisera was tested by their ability to inhibit the effects on immature rats and oestrous rabbits of gonadotropic extracts of horse, ox, sheep, pig and human

pituitaries, human and mare pregnancy serum and human urine of pregnancy and placenta.

Complete "species-specificity" is shown by the reaction of the antisera to gonadotropic extracts of human urine of pregnancy and pregnant mare serum, whereas antisera to similar extracts of pituitary origin exhibit only incomplete "species-specificity". Complete "source-specificity", on the other hand, is only found in the reactions of the antiserum to gonadotropic extracts of pregnant mare serum.

The difficulties encountered in the interpretation of these results and their bearing on the probable occurrence in the pituitary of separate follicle-stimulating and luteinizing hormones and of a gonadotropic antagonist is discussed, and it is concluded that much more evidence is required before such specificity as is shown by antigonadotropic sera can be ascribed to immunological factors.

REFERENCES

Brandt, R. and Goldhammer, H. 1936 *Z. ImmunForsch.* **88**, 79–90.

Collip, J. B. 1934 *Mt Sinai Hosp. J.* **1**, 28–71.

— 1937 *Canad. Med. Ass. J.* **36**, 199–200.

Fevold, H. L., Hisaw, F. L. and Leonard, S. L. 1931 *Amer. J. Physiol.* **97**, 291–301.

Fluhman, C. F. 1935*a* *Proc. Soc. Exp. Biol., N.Y.*, **32**, 1595–6.

— 1935*b* *Amer. J. Obstet. Gynaec.* **30**, 584–9.

Gegerson, H. J., Clark, A. R. and Kurzrok, R. 1936 *Proc. Soc. Exp. Biol., N.Y.*, **35**, 193–5.

Gustus, E. L., Meyer, R. K. and Dingle, J. H. 1935 *Proc. Soc. Exp. Biol., N.Y.*, **33**, 257–61.

Parkes, A. S. and Rowlands, I. W. 1936 *J. Physiol.* **88**, 305–11.

Rowlands, I. W. 1936 *J. Physiol.* **88**, 298–304.

— 1937*a* *Proc. Roy. Soc.* B, **121**, 517–32.

— 1937*b* *J. Physiol.* **90**, 12 *P*.

— 1937*c* *Proc. Roy. Soc.* B, **124**, 492–503.

Rowlands, I. W. and Parkes, A. S. 1937 *Lancet*, April 17, pp. 924–6.

Thompson, K. W. and Cushing, H. 1937 *Proc. Roy. Soc.* B, **121**, 501–17.

Twombly, G. H. 1936 *Endocrinology*, **20**, 311–17.

Zondek, B. and Sulman, F. 1937 *Proc. Soc. Exp. Biol., N.Y.*, **36**, 712–17.

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END OF THE ONE HUNDRED AND TWENTY-FOURTH VOLUME (SERIES B)

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

In accordance with a resolution of Council, summaries or abstracts of papers are to be published as soon as practicable. The publication of such abstracts in no way indicates that the papers have been accepted for publication in any fuller form. These abstracts will be issued for convenience with the "Proceedings of the Royal Society of London" but do not form a part of the "Proceedings".

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19 November 1937

The Temperature Variation of the Work Function of Clean and of Thoriated Tungsten

By A. L. REIMANN

(*Communicated by R. H. Fowler, F.R.S.—Received* 3 *June* 1937)

Measurements were made of the contact potential difference between a constant source of electrons and a neighbouring tungsten filament collecting space-charge-limited electron current, whose condition was varied. This filament was either clean or covered with a layer of thorium atoms, and, in addition, it was held at various temperatures. In this way direct information was obtained concerning the rates at which the work functions of clean and fully activated thoriated tungsten (W-Th) vary with temperature. Within the limits of experimental uncertainty there is no difference between

the temperature coefficients of the two work functions. The temperature coefficient found is about that which would be required to account for the observed value of the thermionic constant A of clean tungsten on the assumption of practically perfect transmission. It must be concluded that the factor (of the order of 10–20) by which the A of W-Th is less than that of clean tungsten is due to internal reflexion of electrons at the W-Th surface.

The Scattering of Wireless Waves in the Ionosphere

By T. L. Eckersley

(*Communicated by W. H. Eccles, F.R.S.—Received* 11 *June* 1937)

Application of the Phase Integral Method to the Analysis of the Diffraction and Refraction of Wireless Waves round the Earth

By T. L. Eckersley and G. Millington

(*Communicated by W. H. Eccles, F.R.S.—Received* 11 *June* 1937)

Relaxation Methods applied to Engineering Problems

II—Basic Theory, with applications to Surveying and to Electrical Networks, and an extension to Gyrostatic Systems

By A. N. Black, B.A. and R. V. Southwell, F.R.S.

(*Received* 22 *June* 1937)

The method of systematic relaxation, originally devised for the calculation of stresses in frameworks, has since been extended to other elastic systems. This paper shows that its basis is the principle of minimum potential energy, and thereby demonstrates its convergence as applied not only to elastic problems, but to any problem for which a minimal theorem exists. Examples treated are the adjustment of errors in surveying and the partition of electric current in non-inductive networks.

Finally, networks characterized by self-induction and/or capacity are discussed as examples of "gyrostatic systems", which do not present minimal problems of the standard type. Here too convergence can be demonstrated, and a numerical example shows that no special difficulty is presented.

The Seiches in a Strait connecting Two Seas

By G. R. Goldsbrough, F.R.S.

(*Received* 29 *June* 1937)

The problem studied is that of the natural vibrations of the water in a strait connecting two open seas. The shores of the strait are the two branches of a hyperbola and a certain law of depth is chosen. It is shown that such a system has free modes of four distinct types characterized by symmetry about both axes, asymmetry about the conjugate axis, asymmetry about the transverse axis and asymmetry about both axes, respectively. In each case there is an infinite number of distinct modes. The waves diminish rapidly in amplitude as the channel widens. The nodal

lines are members of the same family of confocal ellipses and hyperbolas of which the shores are a member.

Some of the lower modes are worked out completely for special cases. But simpler approximations are given covering all the forms where the eccentricity of the bounding hyperbola is not too small.

On the Torsion of Conical Shells

By R. V. Southwell, F.R.S.

(*Received* 30 *June* 1937)

By imagining a flat plate to be distorted by shearing stresses in its own plane, and then with shear stresses operative to be bent into the appropriate shape, the Bredt-Batho formula for the stresses produced by torsion in non-circular tubes is obtained and extended to shells having the forms of non-circular cones (tapered tubes). The strains are also deduced, and the results for cylindrical tubes conform with what are given by the classical theory of Saint-Venant, both as regards the twist and the warping of cross-sections out of their planes. In thin conical shells it is shown that warping will be replaced by a strain which alters the shapes of cross-sections, for the reason that it would involve extensions, which occurring in a sheet of negligible thickness would violate the principle of minimum strain-energy. In cylindrical tubes, either type of strain is possible according to a theory in which the thickness is neglected: warping occurs in fact, because unlike the other alternative it involves no flexural stresses.

Part II is concerned with the question, whether Saint-Venant's celebrated theory of torsion can be extended so as to include *thick* tubes and rods having the forms of non-circular cones. It is found (as would be expected from the foregoing results) that except in relation to sections of a special (circular or oval) type no progress can be made by assuming that the shapes of the cross-sections are not altered by torsional strain.

Galvanometer Effects in Bismuth Alloys

By W. Thompson

(*Communicated by A. M. Tyndall, F.R.S.—Received* 3 *July* 1937)

An account is given of experimental observations on the Hall effect and transverse magneto-resistance effect in single crystals of bismuth containing small and known amounts of impurity, up to 1 % of lead and 0·1 % of selenium. Measurements were made at fields up to 22,000 oersted, at temperatures from 20° A. to 290° A., and for three principal crystal orientations. The results are found to be in general agreement with theory, and a hypothesis is advanced to explain the divergencies. It is concluded that the number of free electrons per atom in metallic bismuth is of the order of 3×10^{-5}.

Atomic Hydrogen

By H. G. Poole

(*Communicated by C. K. Ingold, F.R.S.—Received* 6 *July* 1937)

I—The Calorimetry of Hydrogen Atoms

A new type of calorimeter for the measurement of hydrogen atoms is described, depending on the continuous flow principle: water flowing through a copper tube is warmed by the catalytic recombination of atoms on the platinum-plated outer surface of the tube, and the rise of temperature of the water then determines the rate of recombination of atoms. Considerable accuracy is possible, and the sensitivity of the calorimeter is easily varied as required; sensitivities of 0·1–1·0 calories per minute have been used, the range of rates of heat production so far studied being 4–300 calories per minute. Continuous operation and reading over indefinite periods of time are possible, very small corrections due to losses are required, and the lag in attaining equilibrium after large changes in the rate of supply of atomic hydrogen is of the order of 1 to 2 min.

The spectroscopic value of Beutler for the dissociation energy of hydrogen (102,720 cals./g.-mol.) is adopted; after allowance for the heat capacities

of the molecules and atoms this leads to 103,680 cals./g.-mol. for the heat of dissociation at 300° K. and constant pressure.

The determination of hydrogen atoms by molecular flow gauges is discussed and sources of error which have hitherto been overlooked are pointed out. In particular, the effect of small errors in the measurement of pressure is shown to be large.

II—Surface Effects in the Discharge Tube

The output of atomic hydrogen from several different discharge tubes of silica and pyrex has been measured calorimetrically at one-minute intervals. The curves obtained show that very large changes in atom output with time, even in a few minutes, may occur with discharge tubes depending only on a water-on-glass or water-on-silica film for de-activation of the tube wall as catalyst for the recombination of atoms, and that the behaviour of such tubes depends on their past history. The conclusion is reached that destruction of the protective water film occurs during intense discharges, and that readsorption of water takes place during rest periods and sometimes during weak discharges; the increasingly rapid decay of output for successive high intensity discharges indicates that a new water film is less firmly held than an old film.

Constant and reproducible output is obtained by the use of a metaphosphoric acid lining inside the discharge tube.

III—The Energy Efficiency of Atom Production in a Glow Discharge

The efficiency of the glow discharge system for the production of hydrogen atoms has been determined as the number of dissociated molecules received at the calorimeter per unit energy (electron-volt) supplied to the positive column. This efficiency, μ_c, has been found to vary with the power input to the positive column, W, with the volume rate of flow of gas, U_0, and with the pressure; for any given pressure, the behaviour of the discharge tube is satisfactorily represented by the empirical equation

$$\frac{1}{\mu_c} = \frac{1}{\mu_0}\left(1 + \frac{k}{U_0}\right) + \left(\frac{a}{U_0} - b\right) W$$

in which μ_0, k, a and b are constants depending on the pressure.

Analysis of the conditions in the discharge tube and delivery tube has indicated the reasons for the above relationship; and has led to the determination of the Emeléus-Lunt-Meek efficiency, η_0, i.e. the sum of the efficiencies for the excitation of the two states, $^3\Sigma_u^+$ and $^3\Sigma_g^+$, leading to dissociation of the molecule, over the pressure range 0·10–1·35 mm. The variation of η_0 as the pressure is changed is in satisfactory agreement with the theoretical values, provided that a theoretically permissible correcting factor of 2·6 is applied to the probability cross-section for $^3\Sigma_u^+$ excitations as calculated quantum-mechanically by Massey and Mohr. The velocity constant for the first order, wall-catalysed recombination of atoms has been determined from the same data, and hence the adhesion coefficient γ for a water-on-phosphoric acid film, i.e. the fraction of the total number of collisions of atoms with the walls which lead to the loss of an atom; γ is found to be $3{\cdot}9 \times 10^{-5}$.

In addition, a general equation for the flow of atoms and molecules through a tube has been developed.

Photochemical Polymerization of Methyl Methacrylate Vapour—I

By H. W. Melville

(*Communicated by E. K. Rideal, F.R.S.—Received* 12 *July* 1937)

The kinetics of the photochemical polymerization of methyl methacrylate have been investigated with the following results. Polymerization occurs nearly quantitatively to a white solid, if the radiation is absorbed by the $COOCH_3$ group in the molecule, i.e. $\lambda > 2200$ A. Light of shorter wavelength absorbed by the double bond leads to decomposition of the molecule.

After a sufficient time of illumination, the polymer grows continuously in the dark for several days. This steady growth is proportional to the area of the tube illuminated, to the methacrylate pressure and decreases with increasing temperature. It can be stopped by means of H atoms and I_2 molecules; it is unaffected by oxygen, ethylene and acetylene. The polymer is depolymerized to the monomer on heating to 300° C. *in vacuo.*

Polymerization can also be initiated by hydrogen atoms, the velocity of which is greater than that of the normal polymerization. The rate is independent of methacrylate pressure (above 2 mm.) and hydrogen pressure (above 4 mm.), and is proportional to the square root of the intensity of the light. The mean life of this polymer is *ca.* 10^{-2} sec. It stops growing when two growing polymers collide in the gas.

The mechanism of the growth of the methacrylate polymer is discussed and reasons advanced to explain why one polymer has a long life and the other a short life. The essential difference is believed to be due to a double bond polymerization in the former case and to a free radical polymerization in the latter case.

Photochemical Polymerization of Methyl Methacrylate Vapour—II

By H. W. MELVILLE

(*Communicated by E. K. Rideal, F.R.S.—Received* 12 *July* 1937)

When atomic hydrogen, generated photochemically, is produced in presence of methyl methacrylate vapour, a polymer is formed as a colourless oil, much faster than the normal polymerization mentioned in the previous paper. This polymerization reaction has entirely different characteristics. It occurs wholly in the gas phase and stops immediately the light is cut off. Above pressures of 2 mm. of methacrylate, the rate of polymerization is independent of methacrylate pressure. The rate is also independent of hydrogen pressure above 4 mm. It is proportional to the square root of the intensity of the absorbed light. By using a rotating sector the mean life of the polymer is shown to be less than 10^{-2} sec. A kinetic analysis of the results demonstrates that the polymer stops growing when *two* growing polymer molecules unite.

Considering the above results together with those described in the previous paper, the question of the mechanism of growth of methyl methacrylate polymers is discussed. It is concluded that in the normal photochemical polymerization growth on the surface occurs by the reproduction of a double bond at the end of a polymeric molecule each time a monomer adds on. This accounts for the stability of the polymer, its behaviour toward solvents and ease of thermal depolymerization. The initial gasphase growth of the polymer may, however, proceed through the medium of a free radical polymerization exactly analogous to that occurring in the hydrogen sensitized polymerization.

Dissociation, Recombination and Attachment Processes in the Upper Atmosphere—I

By H. S. W. Massey, Ph.D.

Independent Lecturer in Mathematical Physics,
Queen's University, Belfast

(*Communicated by E. V. Appleton, F.R.S.—Received* 13 *July* 1937)

The probabilities of various collision reactions which occur in the *E* and *F* regions of the ionosphere are considered in general terms. In particular the relative importance of recombination and attachment in effecting electron removal is discussed. As processes in which electrons are detached from negative ions can be expected to go on at a considerable rate (much faster than recombination) and as attachment must also occur very rapidly, the most reasonable representation of the phenomena is to consider the negative ions and electrons as in a dynamical equilibrium adjusting itself rapidly to loss of either by recombination. In this way the usual recombination theory may be retained, even though attachment occurs much more rapidly. A consequence of the theory is that the number of negative ions should considerably exceed the number of electrons in the *E* region as required by the "dynamo" theory of magnetic variations. The low rate of recombination in the *F* region, requiring the density of positive ions to be not much greater than that of the electrons, makes it necessary to suppose that, at these levels, the atmospheric gases present are inert towards electron attachment.

A Transmutation Function for Deuterons

By P. L. Kapur

(*Communicated by R. H. Fowler, F.R.S.—Received* 15 *July* 1937)

A generalization of the method of geometrical optics, commonly known as the Wentzel-Kramers-Brillouin method, is applied to the case of transmutation by deuterons. The treatment of Oppenheimer and Phillips (1935), based on the approximation of a three-body problem, is improved in so far as the wave equation is not assumed to be separable. The generalization gives, in general, an upper limit to the probability of the process, but in the present case it is shown that it gives the correct value. The results of the calculations are given in Table I and figs. 1 and 2. Formulae ((18′) and (21′)) are also obtained which take into account the finite radius of the nucleus. The results are compared with experiments on copper and are found to be in fair agreement, when the binding energy of the deuteron is taken to be 2·25 mV.

A Study of Upper Carboniferous Coals from Western Australia

By C. R. Kent, Ph.D.

(*Communicated by Professor William A. Bone, F.R.S.—*
Received 19 *July* 1937)

Explosion Waves and Shock Waves

V—The Shock Wave and Explosion Products from Detonating Solid Explosives

By W. Payman, D.Sc., Ph.D. and D. W. Woodhead, Ph.D., F.I.C.

(*Communicated by J. F. Thorpe, F.R.S.—Received* 20 *July* 1937)

The mode of expansion of the disturbance produced when a cartridge of explosive suspended freely is detonated has been studied by means of

Schlieren photography, using cameras of both instantaneous and continuous types. The records show that there are three phases in the life of the disturbance produced.

The first phase is the detonation of the cartridge. This is comparatively short (about 3×10^{-5} sec. in a 113 g. cartridge of celmonite). The disturbance then may be regarded as arising solely from the side of the cartridge and is very nearly spherical; the shock wave is smooth and precedes the front of the gaseous products by a fraction of a centimetre.

The second phase is longer and may last until the disturbance has travelled 100–125 cm. (6×10^{-4} sec. with a 113 g. cartridge of celmonite). During the first half of this period the gaseous products are for the most part luminous and follow the shock wave at a gradually increasing distance. The velocities of both wave and products may be greater than the rate of detonation of the cartridge. Many characteristic prominences due to individual solid particles and congregations of particles appear in the wave front. The gaseous expansion from each end of the cartridge is far more vigorous than that from the side, with the result that axial distensions in the form of the wave appear. These deformations from the spherical are accentuated and prolonged by the shower of particles, the density of the shower being clearly greatest axially with the cartridge. In the second half of this period the disturbance is no longer "maintained". The velocity of the gaseous products falls rapidly and the products become mixed with air through turbulence to an increasing extent. Simultaneously, the masses of particles producing the prominences in the wave front become dispersed and, as individual particles, no longer affect materially the progress of the wave.

The third and last phase begins when all prominences have disappeared; the wave becomes smooth again and, when travelling at about 500 m./sec., rapidly assumes the spherical form. The gaseous products are at atmospheric pressure and free to drift. Dispersed particles precede the wave over several metres and sooner or later fall to the ground. The wave spreads with a gradually falling velocity until it ultimately degenerates into a sound wave.

Some Conditions Governing the Solubility of Iron

BY L. H. N. COOPER, PH.D., F.I.C.

Assistant Chemist at the Plymouth Laboratory of the Marine Biological Association

(*Communicated by W. R. G. Atkins, F.R.S.—Received* 26 *July* 1937)

Existing data on the solubility products of ferrous and ferric hydroxides and on the ion, $FeOH^{++}$, have been used to calculate the maximum activities of Fe^{++}, $FeOH^{++}$ and Fe^{+++} which may exist in sea water.

The maximum activity of ferrous ion is controlled both by the activity of the ferric ion and by the oxidation-reduction potential of the system.

The maximum activity of the three ions at equilibrium in sea water are presented in Table VII. Other tables show equilibrium conditions at a range of oxidation-reduction potentials and hydrogen-ion concentrations.

The total quantity of iron in true solution in water after equilibrium has been attained does not exceed

3×10^{-8} mg. iron per cubic metre at pH 8·5
4×10^{-7} ,, ,, ,, 8·0
4×10^{-5} ,, ,, ,, 7·0
5×10^{-3} ,, ,, ,, 6·0

and of this the greater part consists of ferrous and $FeOH^{++}$ ions. In more acid solutions ferrichloric acids may have to be taken into account.

These values apply to iron in sea water, in natural waters and in many physiological fluids.

On the Equations of Electromagnetism

I—Fundamental Identifications

BY E. A. MILNE, F.R.S.

(*Received* 26 *July* 1937)

The paper offers a purely kinematic formulation of the phenomena of electromagnetism. It proceeds by first examining the most general type of external force after gravitational forces which are encountered in the study of the dynamics of a particle in the presence of the substratum or smoothed-out universe. Such external forces involve mention of 6-vectors, and the form of the resulting force is obtained in a form holding good for all epochs and for all distances from the observer. For the present epoch and at small distances it reduces to the Larmor-Lorentz formula for the ponderomotive force on a moving charge. A scalar multiplier emerges in the treatment which is afterwards identified as charge. A 6-vector is then derived by appropriate differentiation of an undetermined scalar with regard to the co-ordinates of two events, one being the source of the field and the other the event of the measurement of the field. With this it is possible to obtain the relativistic equations of motion of two charged particles in one another's presence, and to deduce the associated energy equation. By requiring the radiation to vanish when the accelerations vanish, it is found possible to fix all undetermined scalars completely. It is then possible to evaluate the "field" due to a given charge in given motion, at a given external point, as measured by the force on a test-charge in given motion, and by comparing the theoretically derived formulae with the observed experimental laws to identify the various symbols introduced. The inverse square law of Coulomb is obtained in exact relativistic form. But the law expressing the magnetic field at a distance r from a given charge e_2 moving with a given velocity $\mathbf{V}_2$ relative to a fundamental observer O is found to contain mention of the velocity $\mathbf{V}_1$ of the test-charge measuring the field; in detail, the classical Biot and Savart law $\mathbf{H} = (e_2/c)\, \mathbf{V}_2 \wedge \mathbf{r}/|\mathbf{r}|^3$ is found to be replaced by

$$\mathbf{H} = (e_2/c)\, \tfrac{1}{2}(\mathbf{V}_1 + \mathbf{V}_2) \wedge \mathbf{r}/|\mathbf{r}|^3.$$

The two agree for $\mathbf{V}_1 = \mathbf{V}_2$, but the new formula gives half the classically calculated field for $\mathbf{V}_2 = 0$, as required by Uhlenbeck and Goudschmidt and as found by a different method by L. H. Thomas. Our result is of more than mere numerical significance, as it compels the abandonment of the concept of a magnetic field as something independent of the circumstances of its measurement. This will be more fully developed in Part II.

Secular factors in the laws of electromagnetism are considered, and the results applied to the simple Bohr atom and to Dirac's cosmological relation. It is shown that in kinematic measure the radius of an atom is proportional to t, tending to zero as $t \to 0$. It is also shown that Dirac's relation does not require the creation of matter.

On the Equations of Electromagnetism

II—Field Theory

By E. A. Milne, F.R.S.

(*Received* 25 *July* 1937)

Following the mathematical treatment of the preceding paper, a physical discussion is given in which it is shown that the concept of an electromagnetic field (**E**, **H**) as existing in free space independent of the circumstances of the test-charge used to measure it must be abandoned. Instead it is replaced by the concept of a pair (**E**, **H**) which depend on the velocity **V** of the test-charge at the point concerned used to measure it. Nevertheless "field identities" are found which are satisfied at each event by the (**E**, **H**) as measured there; these identities are explicitly independent of the velocity **V** of the test-charge employed. These are stated as equations (12), (13), (29), (30). Two of them coincide with two of Maxwell's equations, namely those expressing the non-existence of isolated magnetic poles and Faraday's law of electromagnetic induction. The remaining two are modifications of Maxwell's other two equations for free space. They imply wave-propagation of **E**, **H**, and admit the existence of a vector-potential.

With the abandonment of the concept of a field as existing in free space independent of the velocity of the test-charge used to measure it, the expression of electromagnetic energy as a volume integral must be abandoned also. Instead we have an expression for the electromagnetic energy associated with the different pairs of charges present in the field, calculated mechanically. The sum of this and the mechanical energies of the moving massive particles varies with the time according to a linear function of the accelerations of the charged particles in one another's presence. The latter vanishes in certain well-defined circumstances, or has a mean value zero, and this gives rise to the phenomenon of non-radiating, i.e. "stationary", states. Radiation occurs in certain cases of accelerated motion, but not for periodic systems. The rôle of the field is then the same as that put forward by Bohr, Kramers and Slater, namely that energy is not located in the field but that the field is the mechanism for conveying energy from one set of charged particles to another.

A unified treatment of gravitational and electromagnetic phenomena is briefly sketched, and the energy formula obtained.

Structure of Stretched Rubber

By C. J. B. Clews and F. Schoszberger

(*Communicated by Sir William Bragg, P.R.S.—Received* 26 *July* 1937)

A picture of micellar structure is developed which is intermediate between the two formerly proposed theories. The micelles are of lath-shaped form, and possess, on account of their shape and of their transverse bindings, a definite individuality, even though the "Hauptvalenzketten" themselves extend beyond the sphere of single micelles.

Rubber films, obtained from dilute solutions in various solvents, have been examined to determine a possible influence on "higher orientation". The X-ray photographs were taken on a fibre camera.

All films derived from these solutions show "higher orientation" on extreme extension to 700 %. It is therefore established that the capacity for building up micelles in the way described is an intrinsic property of the "Hauptvalenzketten".

The Continuous Absorption Spectrum of Methyl Bromide and its Quantal Interpretation

By Phyllis Fink and C. F. Goodeve

The Sir William Ramsay Laboratories of Inorganic and Physical Chemistry, University College, London

(*Communicated by F. G. Donnan, F.R.S.—Received* 26 *July* 1937)

The extinction coefficients of methyl bromide have been measured over the range 35,000 to 52,000 cm.$^{-1}$. Quantal methods previously applied to the interpretation of continuous absorption bands of diatomic molecules have been extended to the interpretation of the band of methyl bromide. The upper potential energy curve has been calculated and the extinction coefficient curve resolved into the parts arising from molecules in various vibrational levels. The analytical method shows the construction of thresholds of continuous absorption.

Hyperfine Structure and Nuclear Moments of Aluminium

By D. A. Jackson and H. Kuhn

Clarendon Laboratory, Oxford

(*Communicated by F. A. Lindemann, F.R.S.—Received* 28 *July* 1937)

The hyperfine structure of the resonance lines of aluminium was investigated by means of the absorption in an atomic beam of aluminium. The line $3\,^2P_{\frac{1}{2}}$–$4S_{\frac{1}{2}}$ was found to possess three components at $-0{\cdot}048$, $0{\cdot}000$ and $+0{\cdot}048$ cm.$^{-1}$ of nearly equal intensity. The line $3\,^2P_{\frac{1}{2}}$–$3\,^2D_{\frac{3}{2}}$ possessed two components of separation $0{\cdot}066$ cm.$^{-1}$, the intensity ratio of which was measured to a high degree of accuracy; the value found was $1{\cdot}21:1$, the component of longer wave-length being the stronger.

From the structure of the line $3\,^2P_{\frac{1}{2}}$–$4S_{\frac{1}{2}}$ it follows that the levels $4S_{\frac{1}{2}}$ and $3\,^2P_{\frac{1}{2}}$ are both split into two levels, of separation $0{\cdot}048$ cm.$^{-1}$ (the greater splitting of the line $3\,^2P_{\frac{1}{2}}$–$3\,^2D_{\frac{3}{2}}$ is due to a small unresolved, inverted structure of the level $3\,^2D_{\frac{3}{2}}$ which is probably caused by perturbation by the term $3s\,3p^2\,{}^2D_{\frac{3}{2}}$). The observed intensity ratio of the components of the line $3\,^2P_{\frac{1}{2}}$–$3\,^2D_{\frac{3}{2}}$ gives a value $\frac{9}{2}$ for the nuclear spin, and the splittings of the levels $4S_{\frac{1}{2}}$ and $3\,^2P_{\frac{1}{2}}$ give values $4{\cdot}1$ and $3{\cdot}6$ nuclear magnetons respectively for the magnetic moment of the nucleus according to the formulae of Goudsmit.

The lines $4S_{\frac{1}{2}}$–$5\,^2P_{\frac{3}{2}}$ and $4S_{\frac{1}{2}}$–$5\,^2P_{\frac{1}{2}}$ were observed in emission and were found to be doublets, only just resolvable on account of their Doppler width, of separation about $0{\cdot}05$ cm.$^{-1}$ and of intensity ratio (when corrected for overlapping) about $1{\cdot}23:1$, the component of longer wave-length being the stronger. This structure is in complete agreement with the interpretation of that observed in the resonance lines.

This result is in disagreement with the contents of a preliminary note by Ritschl in which, from an observed doublet structure of the arc lines 3057 and 3050 and the spark line 2669, he suggested a nuclear spin of $\frac{1}{2}$; an investigation of the structure of these lines showed that the observed doublet structures do not represent a complete resolution.

Penetration into Potential Barriers in Several Dimensions

By P. L. Kapur and R. Peierls

(*Communicated by R. H. Fowler, F.R.S.—Received* 30 *July* 1937)

The method of geometrical optics is discussed in application to the penetration of particles into regions of negative kinetic energy. It is shown that in the case of problems in more than one dimension which cannot be separated, it is generally possible to derive an upper limit for the probability of penetration, and that in special cases the method gives the actual value of this probability.

The Geometrical Representation of Milne's Kinematical Relativity

By W. H. McCrea

(*Communicated by E. T. Whittaker, F.R.S.—Received* 30 *July* 1937)

It is shown that the assumptions on which Milne's kinematical relativity rests may be translated into the axioms of incidence of projective geometry, together with the usual axioms of order and continuity. The analytical expression of Milne's theory corresponds then to the metrical expression of the geometrical properties achieved in the usual way by the use of an Absolute Quadric. This throws light, in particular, on Milne's introduction of the Lorentz transformation. It also exposes certain restrictions imposed by his assumptions, as compared, for example, with those of general relativity. Further, it makes explicit the degree of arbitrariness allowable in the choice of a "geometry" to represent the properties of the system.

On Anomalous Vibrational Spectra

By M. Blackman, *Trinity College, Cambridge*

(*Communicated by R. H. Fowler, F.R.S.—Received* 4 *August* 1937)

The equation of motion of an elastic continuum is investigated, and it is shown that the continuum becomes unstable when the elastic constants obey certain relations. The vibrational spectrum of lattices for which these relations hold is shown to undergo a characteristic change, the density of normal vibrations going over into the one-dimensional or into the two-dimensional form. The significance of this is considered from the point of view of the specific heat and the vibrational spectrum of actual crystals; it is found that no actual crystals are near enough to the critical regions for the effect of the approach to be apparent in the specific heat, but in some cases this should be noticeable in the vibrational spectrum.

Refractive Dispersion of Organic Compounds

IX—Optical Exaltation in Unsaturated Hydrocarbons containing Conjugated Double Bonds

By (the late) T. M. Lowry, F.R.S. and C. B. Allsopp, M.A., Ph.D.
Laboratory of Physical Chemistry, The University, Cambridge

(*Communicated by R. G. W. Norrish, F.R.S.—Received* 21 *August* 1937)

Optical exaltation appears to be exhibited by a system of two conjugated ethylenic double bonds when these form part of an open chain (e.g. 2 : 4-hexadiene) but not when they are built into a ring (e.g. 1 : 3-*cyclo*hexadiene). The ultra-violet absorption bands of conjugated ring systems are displaced towards lower frequencies relative to those of the corresponding open-chain systems. These effects of configuration on the optical properties of a conjugated system are discussed.

Crystal Growth from Solutions

BY W. F. BERG, PH.D. (BERLIN), PH.D. (MANCHESTER)

(*Communicated by W. L. Bragg, F.R.S.—Received* 21 *August* 1937)

An optical method of measuring the concentration distribution round a growing crystal is described. The flow of molecules at every point of the crystal surface can be determined. The results obtained on sodium chlorate are:

1—The concentration is not constant all over the crystal surface, but is highest at the corners.

2—One has to assume the existence of an adsorbed layer on the crystal surface, and that a lateral flow of molecules takes place in this layer.

3—There is a hitherto unexplained effect influencing the transition of molecules from the solution into the different parts of the adsorbed layer. The influx is smaller at the corners than at the centres of the faces, although the concentration of the solution is higher on the corners.

Measurements of Range and Angle of Projection for the Protons produced in the Photo-Disintegration of Deuterium

BY J. CHADWICK, F.R.S., N. FEATHER AND E. BRETSCHER

(*Received* 8 *September* 1937)

Measurements of range and angle have been made on the tracks of 62 protons produced in an expansion chamber containing a deuterium-rich gas and irradiated by the γ-rays from a radiothorium source. These measurements have been analysed in detail and critically discussed. It is concluded that the determinations of range indicate a binding energy of $2{\cdot}25 \pm 0{\cdot}05 \times 10^6$ e-volts for the deuteron and that the angular distribution implies a considerable preponderance of the photoelectric over the photomagnetic type of disintegration with the radiation employed ($h\nu = 2{\cdot}62 \times 10^6$ e-volts).

Investigations on Magne-crystallic Action
V—Para-Magnetic Salts of the Rare Earth and the Iron Groups of Metals

By K. S. Krishnan and A. Mookerji

(*Communicated by Sir Venkata Raman, F.R.S.— Received* 9 *September* 1937)

On the Forces acting between Atoms and Ions and the Physical Properties of Matter in Bulk

By J. A. Wasastjerna

(*Communicated by W. L. Bragg, F.R.S.—Received* 9 *September* 1937)

A method is developed according to which it is possible to analyse, by the aid of accessible experimental data, the question of the dependence of the potential energy on the interatomic distance for atoms and ions with closed shells. The results of the analysis have been made the foundation for a theoretical calculation of a number of physical properties of crystals. In many instances the results of the calculations can be compared with experimental data. In other instances information is obtained concerning data which have previously been entirely unknown or about which great uncertainty has prevailed. Finally, a theoretical interpretation is given to the empirical results. The experimental values of the van der Waals forces both for rare gases and ionic crystals are about twice as large as the theoretical ones, and there is thus a rather serious discrepancy.

Studies of the *E* Region of the Ionosphere

By J. E. Best, F. T. Farmer and J. A. Ratcliffe

(*Communicated by E. V. Appleton, F.R.S.—Received* 9 *September* 1937)

Statistical Mechanics of the Adsorption of Gases at Solid Surfaces

By F. J. Wilkins

(*Communicated by R. H. Fowler, F.R.S.—Received* 20 *September* 1937)

The generalized adsorption isotherm is shown to be

$$Cg/Ca = \frac{fg(T)\,B_{Ng}(T)}{fa(T)\,B_{Na}(T)}\,e^{-\phi/RT+\left\{Ng\frac{\partial}{\partial_{Ng}}\log B_{Ng}(T)-Na\frac{\partial}{\partial_{Na}}\log B_{Na}(T)\right\}},$$

where Ca and Cg are the molecular concentrations in the adsorbed and gaseous phases respectively; ϕ is the adsorption potential and $fg(T)$, $fa(T)$, $B_{Ng}(T)$ and $B_{Na}(T)$ are partition functions. These functions have been evaluated for three special cases in which the adsorbed phase is (*a*) a three dimensional gas, (*b*) a gas of two dimensional lateral mobility whose molecules vibrate in a plane perpendicular to the plane of lateral mobility, and (*c*) a group of Planck oscillators with three degrees of freedom.

The relation between ϕ and q, the differential heat of adsorption, is deduced and it is shown that the common practice of calculating from the equation

$$(\partial \log p/\partial T)_x = -q_x/RT^2$$

is erroneous. The correct equation is

$$(\partial \log p/\partial T)_{(x/\alpha d)} = -q/RT^2,$$

where $(x/\alpha d)$ is the concentration in the adsorbed layer.

The Absorption of Argon, Nitrogen and Oxygen on Smooth Platinum Foil at Low Temperatures and Pressures

By F. J. Wilkins

(*Communicated by R. H. Fowler, F.R.S.—Received* 20 *September* 1937)

The adsorption of argon, nitrogen and oxygen on a smooth platinum foil has been measured at various temperatures between 77 and 193° K. over the pressure range of 5×10^{-3} to 2×10^{-1} mm. It is found that the deviations from the Langmuir adsorption isotherm are greater the lower is the temperature. The values α of the fraction of the apparent surface covered by the gas at saturation is unity only for argon at 77° K. In all other experiments α was much less than unity. Further, the rate of decrease of α with increase of temperature is much more rapid than is given by Zeise's linear equation and can be expressed approximately by

$$\alpha = ce^{-\beta/T},$$

where c and β are constants. After a discussion of the results, using the virial adsorption isotherm, the failure of the Langmuir adsorption isotherm at low temperatures is attributed to the intermolecular forces of attraction which exert a pronounced effect on the amount of gas adsorbed.

The adsorption potentials of argon and nitrogen are calculated to be approximately 5000 and 5500 cal./g. mol. respectively. These compare with the corresponding values calculated from quantum mechanics of 3200 and 1500 cal./g. mol. The possible reasons for the discrepancy are discussed.

An Attempt to Detect the Passage of Helium through a Crystal Lattice at High Temperatures

By Lord Rayleigh, F.R.S.

(*Received* 20 *September* 1937)

It is known that helium will pass through silica glass, even at ordinary temperatures. A plate 1 mm. thick and 1 sq. cm. area will pass about $2{\cdot}5 \times 10^{-1}$ cu. mm./day. In this case the helium probably passes through submicroscopic channels or cracks in the material. No single crystal, so far as has been ascertained, will pass measurable quantities of helium through the lattice at ordinary temperatures.

Mica has now been tested up to a temperature of 415° C. The amount of helium passed, by a plate 1 mm. thick and 1 sq. cm. area, if any, is certainly less than 7×10^{-5} cu. mm./day.

The Behaviour of an Osglim Lamp
III—Osglims in Parallel forming Models of Reciprocal Inhibition

BY LEWIS F. RICHARDSON, F.R.S.

(*Received* 21 *September* 1937)

Two osglim lamps wired in parallel with one another can be so arranged that the glowing of either inhibits the other. This arrangement is called a "distraction circuit". The lamps are connected through suitable resistances to a battery. An extra stimulus is required to start the extinct lamp. But if a condenser is suitably connected, the apparatus becomes entirely automatic, one or other lamp flashing in a random sequence, but never both lamps together. Although the glowing gas is quite unlike anything in the nervous system, it seems probable that the abstract time-relations of the distraction circuit may be similar to those of a number of psychological and physiological phenomena, such as distraction, or alternations of perspective in line drawings, or reciprocal inhibition.

Experiments with these distraction circuits provide many delicate tests of theories of the osglim lamp. It is shown that the negative slope of the equilibrium potential $\phi_e(u)$ taken in conjunction with the approximate intrinsic equation

$$\phi = \phi_e(u) + b\frac{d\log u}{dt},$$

in which u is current, t time and b is a positive constant, together suffice to explain many of the observed effects; but these hypotheses are inadequate to account for other observations, which however could be explained by the presence of a term of higher order, such as $kd\phi/dt$, where $k \geqslant 4\times 10^{-5}$ sec.

The Production of Neutrons by Bombardment of Beryllium with α-Particles

By T. Bjerge

Institute of Theoretical Physics, Copenhagen

(*Communicated by N. Bohr, For. Mem. R.S.—Received* 21 *September* 1937)

In the energy spectrum of the neutrons from beryllium bombarded with α-particles there are present some groups with energies of several million electron-volts. These are accounted for by the process

$$^{9}_{4}\mathrm{Be} + {}^{4}_{2}\mathrm{He} \rightarrow {}^{12}_{6}\mathrm{C} + {}^{1}_{0}n. \tag{1}$$

The $^{12}_{6}\mathrm{C}$ may be left in an excited state and afterwards emit a γ-quantum.

There is also, as shown by Auger and others, a neutron group of energies of the order of *c.* 100,000 e-volts. Auger gave reasons for supposing that these neutrons are created in a process, where the incident α-particle first transfers some energy to the $^{9}_{4}\mathrm{Be}$, which then disintegrates in the following way:

$$^{9}_{4}\mathrm{Be} \rightarrow 2\,{}^{4}_{2}\mathrm{He} + {}^{1}_{0}n. \tag{6}$$

This view is supported by the ideas of Bohr on nuclear reactions. The present paper describes experiments which give the yield of the faster and slower neutron group and of the γ-rays as a function of the α-particle energy. It is shown that the slower group is not accompanied by γ-rays, and that it begins to be emitted at the α-particle energy which is just necessary for the process (6) to take place.

Stability of Polyatomic Molecules in Degenerate Electronic States. II—Spin Degeneracy

By H. A. Jahn

(*Communicated by Sir William Bragg, O.M., P.R.S.—*
Received 22 *September* 1937)

It is shown that a polyatomic molecule cannot possess a stable non-inear nuclear configuration in an electronic state having spin degeneracy

unless this degeneracy is the special twofold one which can occur only when the molecule contains an odd number of electrons. Instability caused by the spin alone is shown to be of secondary importance compared with the orbital effects discussed in a previous paper. Table I gives the irreducible two-valued representations of all the point groups and will be useful in discussion of the electronic states of polyatomic molecules containing an odd number of electrons with spin.

Radioactive Nodules from Devonshire

By M. Perutz

(*Communicated by J. D. Bernal, F.R.S.—Received* 28 *September* 1937)

A Second-Order Focusing Mass Spectrograph and Isotopic Weights by the Doublet Method

By F. W. Aston, F.R.S.

(*Received* 29 *September* 1937)

Optical Activity in Ketones. The Rotary Dispersion and Circular Dichroism of *m*-Methyl *Cyclo*hexanone and of Pulegone in their Ketonic Absorption Bands

By the late T. M. Lowry, F.R.S., Delia M. Simpson
and C. B. Allsopp

(*Communicated by R. G. W. Norrish, F.R.S.—Received* 1 *October* 1937)

Molecular extinction coefficients, circular dichroism and rotatory dispersion are recorded for *m*-methyl *cyclo*hexanone and for pulegone in the region of their ultraviolet absorption bands. Analysis of the rotatory dispersion on the basis of the observed circular dichrosim shows that the

partial rotation due to the ketonic absorption band of methyl *cyclo*hexanone is much greater than that of the single asymmetric carbon atom, and is opposite in sign. A similar result is obtained for pulegone, despite the conjugation of carbonyl and ethylenic double bonds. By comparison with the data for other optically active ketones containing more than one asymmetric carbon atom, it is concluded that in general the direct contribution of each asymmetric centre is itself small relative to that of the chromophoric group. In the light of Mulliken's analysis of the electronic configurations of aldehydes, the optical activity of the carbonyl group is now attributed to the lone-pair electrons of the oxygen atom, and the theory of "induced dissymmetry" is reinterpreted. A possible function of lone-pairs in producing optical activity in other types of compound is discussed.

On the Penetrating Component of Cosmic Radiation

By H. J. Bhabha

(*Communicated by R. H. Fowler, F.R.S.—Received* 4 *October* 1937)

An analysis of the experimental data is carried through to show that a "break down" theory for radiation loss of electrons cannot explain (1) the latitude effect at sea level from latitudes of 35 to 50°, (2) the large number of particles found at sea level in the difference curves of Bowen, Millikan and Neher for charged particles of 10^{10} e-volts, (3) the shape of the transition curve for large bursts. All these facts can be explained by assuming that the penetrating component consists of new particles with masses between those of the electron and proton. But in order to explain the energy loss measurements of Blackett and Wilson, one must then assume that these particles suffer large energy losses in addition to the ordinary Bremstrahlung which must vary in different substances as Z rather than Z^2, as for example a change in the rest mass of the particles.

The radiation loss and the pair-creation cross-sections taking screening into account accurately for "heavy" electrons are calculated. The frequency of the production of showers of different sizes by such heavy electrons as also the intensity of electrons in equilibrium with such particles forming a soft component are also calculated, and it is shown that

though both these are somewhat larger in heavier elements, the variation is much less than a Z^2 law would give.

If such heavy electrons can be created as usual in pairs, then a part of the hard component at sea level could consist of heavy electrons of mass $10m$ created by the soft component in the upper atmosphere.

It is shown that there are reasons to suppose that the uppermost layers of the atmosphere are more absorbing than one should expect from their mass. This may be due to the fact that the atoms in these layers are largely ionized.

On the Diamagnetic Susceptibility of Bismuth

By M. Blackman

(*Communicated by R. H. Fowler, F.R.S.—Received* 4 *October* 1937)

An extension is made of the existing theory of the marked variation of the susceptibility of bismuth with field strength at low temperatures (known as the de Haas-van Alphen effect). The object of the investigation is to explain the finer features of the experiments, to account for the order of magnitude of the effect and for the dependence on the orientation of the binary axes. The extension consists in assuming a more general form for the relevant part of the surface of constant energy of the electrons. It is found possible to account for all the features of the susceptibility curves and also to fit the experimental curves fairly well. For this purpose one has to assume that the motion of the electrons is violently anisotropic and that only a small fraction of the conduction electrons contribute to the de Haas-van Alphen effect.

Production and Dissipation of Vorticity in a Turbulent Fluid

By G. I. Taylor, F.R.S.

(*Received* 6 *October* 1937)

When isotropic turbulence is set up in a fluid, e.g. by moving a grid of regularly spaced particles through it, the average vorticity decreases with time;

this decrease is due to viscosity. Recently von Karman has calculated the rate at which vorticity is destroyed by viscosity. His equation involves only quantities which can be measured in a wind tunnel by means of the hot wire technique. These quantities have now been measured in one case, and the rate of decrease in the mean square of the vorticity has also been measured. In this case it is proved that the rate of destruction of vorticity by viscosity is four times as great as the rate at which vorticity disappears. Vorticity is therefore being produced by extension of vortex filaments three times as fast as it is disappearing.

It seems that the stretching of vortex filaments must be regarded as the principal mechanical cause of the high rate of dissipation which is associated with turbulent motion.

The Buckling of Grids of Stringers and Ribs

By H. L. Cox, B.A. and H. E. Smith, B.Sc.

Both of the Engineering Department, National Physical Laboratory

(*Communicated by H. J. Gough, F.R.S.—Received* 6 *October* 1937)

The paper describes a theoretical analysis of the stability of a plane rectangular grid consisting of two sets of parallel members (stringers and ribs) intersecting at right angles, when loaded in compression parallel to one set of members (the stringers). Both sets are composed of a number of similar members of uniform section, spaced uniformly, and the ends of all the members are held in the original plane of the grid. The exact solution of this problem appropriate to certain special edge conditions is obtained both by consideration of the equations of equilibrium and by the Rayleigh-Ritz energy method, and the results are compared with previous solutions obtained by an approximate method.

The Emission Band Spectrum of Chlorine (Cl_2^+).—II

By A. Elliott and W. H. B. Cameron

(*Communicated by S. R. Milner, F.R.S.—Received* 7 *October* 1937)

The Hydrolysis of the Methyl Halides

By E. A. Moelwyn-Hughes

(*Communicated by E. K. Rideal, F.R.S.—Received* 19 *October* 1937)

For a chemical reaction proceeding with measurable rate, the most significant single constant is the energy of activation, found by the familiar method of Arrhenius. The value obtained is, in the majority of known cases, a true constant over the accessible temperature regions. In the case of the catalysed hydrolysis of some complicated molecules, namely the glykosides, it has, however, been found (1934) that the energy of activation falls markedly with a rise in temperature. As the result carried significant theoretical consequences, it became necessary to give an experimental answer to the question as to whether this fall was specific to the acidic hydrolyses of glykosides, or of a more general character. In the present work, we have investigated the uncatalysed and uncomplicated hydrolysis of some very simple molecules, namely the methyl halides, and have found that in these cases also the Arrhenius constant falls as the temperature is raised.

The fall effect seems to be most pronounced in, if not confined to, reactions involving collisions between solute and solvent molecules, and as far as can be judged is always negative. The conclusion is confirmed by collateral evidence on the mutarotation of glucose and the decomposition of oxalic acid.

At least three causes are recognized as responsible for the total effect. They are (1) the distribution of energy among a number of oscillators, (2) the electrostatic interaction of the polar molecules of solute and solvent, and (3) the frequency of the rotation of a solute molecule in the solvent atmosphere.

On the Solution of the Laminar Boundary Layer Equations

By L. Howarth

(*Communicated by L. Bairstow, F.R.S.—Received* 20 *October* 1937)

Part I. The problem of the flow along a flat plate placed edgewise to a steady stream, when a retarding pressure gradient varying linearly as the

distance x from the leading edge of the plate is superposed, is discussed. If y denotes distance measured perpendicular to the plate, a solution is obtained in the form of a power series in x whose coefficients are functions of $y/x^{\frac{1}{2}}$. Differential equations are obtained for these coefficients. Seven of the coefficients have been obtained with reasonable accuracy and the eighth and ninth roughly. Unfortunately it appears that about eight more terms are required to carry the solution to the point of separation; the work involved in their determination is prohibitive. Two approximate methods have been developed for determining the error when the first seven terms of the series are used as an approximation. These methods lead to the determination of the point of separation and are in agreement as to its position. If b_0 is the velocity at the edge of the boundary layer at the leading edge of the plate and b_1 is the velocity gradient, separation is found when $b_1x/b_0 = 0{\cdot}120$.

Part II. A method is developed for the solution of the boundary layer equations in any retarded region. It is obtained by replacing the velocity distribution at the edge of the boundary layer by a circumscribing polygon of infinitesimal sides and applying the preceding solution to each of these sides, making the momentum integral continuous at each vertex. The problem is thereby reduced to the solution of a first order differential equation.

The Theory of the Photolysis of Silver Bromide and the Photographic Latent Image

By R. W. Gurney and N. F. Mott, F.R.S.

(*Received* 21 *October* 1937)

An attempt is made to explain the photolysis of silver halides in terms of the concepts of atomic physics. The mechanism by which the silver atoms formed by the light coagulate to form specks of metallic silver is discussed. The ideas used in this discussion are then turned to the photographic latent image, and are shown to account qualitatively for the variation of developed density with temperature, and, for given exposure, with intensity of light. A brief discussion is given of the Herschel effect, and of sensitization by dyes.

Self-consistent Field, with Exchange, for Calcium

By D. R. Hartree, F.R.S. and W. Hartree

(*Received* 27 *October* 1937)

Calculations of the self-consistent field, with exchange, have been made for Ca^{++}, and the results are given.

Solutions of Fock's equations, without allowance for the perturbation of the core by the series electrons, have also been carried out for the $(4s)$, $(4p)$ and $(3d)$ states of Ca^+ and $(4s)^2$, $(4s)\,(4p)$ ^3P and ^1P states of neutral Ca. Energy values for all these states have been calculated and are composed with the observed values; it appears that exchange effects have considerable influence on the relative strengths of binding of a $(4s)$ and a $(3d)$ electron, and so are significant from the point of view of the position of the beginning of the transition group of elements in the periodic table.

Transition probabilities have also been calculated for the $(4s)$–$(4p)$ and $(3d)$–$(4p)$ transitions of Ca^+ and the $(4s)^2$–$(4s)\,(4p)$ singlet transition of neutral Ca; these transitions all give lines of astrophysical interest, and absolute values of transition probabilities may have astrophysical applications.

The Continuous Absorption Spectra of Alkyl Iodides and Alkyl Bromides and their Quantal Interpretation

By D. Porret and C. F. Goodeve

(*Communicated by F. G. Donnan, F.R.S.—Received* 27 *October* 1937)

The extinction coefficient curve for methyl iodide has been analysed according to the method used by Fink and Goodeve, and the existence of two bands has been deduced. The upper potential energy curves corresponding to these bands have been determined.

The extinction coefficients of ethyl iodide and of ethyl and butyl bromides have been determined over a wide range. Influence of the carbon chain has been found to be small. The conditions under which chromophoric groupings retain their individuality have been discussed.

The Measurement of Particle Size by the X-ray Method

By F. W. Jones

(*Communicated by W. L. Bragg, F.R.S.—Received* 23 *October* 1937)

Laue's theory of the broadening of the X-ray diffraction lines by small particles has been discussed and a different value of a constant ω is found. New methods applicable to any type of Debye-Scherrer camera are given for correcting the line breadths for the effect of experimental conditions and the separation of the α-doublet. As an example of the use of these methods a specimen of colloidal gold has been examined and the particle size and shape determined, the procedure in the shape determination having been simplified and made of general use by means of "shape-curves". An attempt has been made to estimate the order of the error introduced by the particle size distribution.

Resolution and Interpretation of the Luminescent Spectra of some Solids at Low Temperatures

By J. Ewles

(*Communicated by R. Whiddington, F.R.S.—Received* 23 *October* 1937)

Resolution of the cathode luminescent spectra of some solids has been obtained at the temperature of liquid air. It is suggested that the spectra may be accounted for as electron vibration bands derived from a neutral metallic atom or an ion of the parent lattice still associated with the lattice. This idea is found to account for the luminescent spectra of a number of solids—some investigated under cathode ray excitation by the writer and some under various modes of excitation by other workers. A possible explanation of some types of phosphorescence is outlined—based on the frequent occurrence of metastable levels in the suggested atomic transitions.

The Infra-red Absorption Spectrum of Methylene Chloride

By C. Cain and G. B. B. M. Sutherland

(*Communicated by R. G. W. Norrish, F.R.S.—Received* 24 *October* 1937)

The Infra-red Absorption Spectrum of Methylene Chloride

By C. Cain and G. B. B. M. Sutherland

(*Communicated by R. G. W. Norrish, F.R.S.—Received* 29 *October* 1937)

The infra-red absorption spectrum of methylene chloride has been examined between 2μ and 12μ with a prism spectrometer. Twenty-six bands have been observed of which sixteen are recorded for the first time. The agreement with earlier experimental work on the vibration spectrum of this molecule is satisfactory. In particular, the existence of a very intense absorption band which cannot be accounted for on the current assignment of fundamental frequencies has been confirmed. The explanation of this absorption band has necessitated a reinterpretation of the whole vibrational spectrum. This has been done using (1) the method of semi-independent groups, (2) an application of the isotope effect in polyatomic molecules of the type YX_4. The latter method is a surprisingly successful way of approximating to the "inter-group" frequencies of a polyatomic molecule and should be capable of wide application in this field.

Two New Echinoidea from the Upper Silurian of Girvan

By E. W. MacBride, F.R.S. and W. K. Spencer, F.R.S.

(*Received* 25 *August* 1937)

The communication deals with two new Echinoids from the Ordovician and a new form interpreted as the first known plated Holothurian.

The new Echinoids are important because they prove that typical Palaeozoic Echinoidea, which characteristically differ in many important characters from later Echinoidea, existed in the Ordovician. Previously it had been supposed that a form with very different build, *Bothriocidaris*, was the typical Ordovician Echinoid. The new forms in consequence have important bearing on the Echinoid ancestry and evolution. They are very well preserved, giving views both of the outer portions of the test and the test interior and are easier to study than any of the later Palaeozoic forms. A rather surprising discovery is that one of the Echinoidea is elongated and bilaterally symmetrical, somewhat like a recent Heart Urchin but with a different plane of symmetry.

At least one Holothurian, *Cucumaria*, is known to have a plated test in an early developmental stage. It is consequently of considerable interest that a plated Holothurian is now known to occur in the early Palaeozoic. The form in most respects has a marked resemblance to an Echinoid and is very good evidence of the view that Echinoids and Holothurians are nearly related. It is suggested that the Echinoids show distinct relationship with the primitive starfish but detailed homologies between starfish, Echinoids and Holothurians are reserved for a later paper.

On Individual Variations in Ability to Acclimatize to High Altitudes

By A. Keys, B. H. C. Matthews, W. H. Forbes,
R. A. McFarland and D. B. Dill

(*Communicated by Sir Joseph Barcroft, F.R.S.—Received* 31 *March* 1937)

1. The International High Altitude Expedition made observations at sea level, 9000, 12,000, 16,000 and 20,000 ft. of the physiological constitution of ten normal subjects. An attempt is now made to correlate these observations with acclimatization.

2. The capability of acclimatization of the members of the party was graded by a questionnaire to which all gave answers as to the deviation in physical and mental performance from their sea level values, of all members of the party. By this classification the members of the party were arranged in order at each altitude.

3. A number of physiological properties were observed at sea level, and at each station. No one of these yielded a good correlation with the classification referred to in paragraph 2 (above), but the whole series taken together with suitable empirical coefficients yield a good correlation with the acclimatization classification. So much so as to encourage the belief that it would thus be possible to predict from sea level measurements whether an individual would acclimatize well or badly.

Electrotonus and Excitation

By H. Rosenberg

(*Communicated by A. V. Hill, Sec.R.S.—Received* 4 *May* 1937)

When an electric current is applied to a nerve a potential difference across the axon membrane is developed. It has previously been suggested that this potential is the intrinsic stimulus, and that the attainment of a certain critical potential beneath the stimulating pole is the threshold condition for excitation. On the basis of the core-conductor theory it is assumed that this potential is measured when the electrotonic potential between the pole and a distant extrapolar point is determined. New experimental evidence with respect to this conception is submitted and related with the physical theory of excitation in nerve (Hill). The main results are:

The voltage-capacity relation for constant subliminal electrotonus fits closely the theoretical curve which represents the data for threshold stimulation with condenser discharges. Similarly the intensity-frequency relation of electrotonus is in agreement with theory and experiment of threshold stimulation by sinusoidal currents within a certain frequency range (300 and 3000 hz.). The time constants derived from observations on electrotonus at the cathode are only about half of those obtained in excitation. If, however, the time constants are determined for catelectrotonus at a short distance from the pole, they are comparable with those found in excitation. Hence the hypothesis is advanced that the establishment of a gradient of critical potential along a finite length of nerve is the threshold condition for excitation. Other possibilities are discussed.

The Effect of Temperature on the Photochemical Bleaching of Visual Purple Solutions

By H. J. A. Dartnall, C. F. Goodeve and R. J. Lythgoe

(*Communicated by Sir John Parsons, F.R.S.—Received* 5 *May* 1937)

Using a new method of extraction, the authors have prepared solutions of visual purple much freer from foreign matter than those obtained

hitherto. Photochemical bleaching curves have been obtained using monochromatic light, with the solutions at various temperatures from 5–60° C. The theoretical treatment of bleaching curves developed in a previous paper (*Proc. Roy. Soc.* A, 1936, **156**, 158) has been extended to allow corrections to be made for the thermal bleaching occurring at the higher temperatures. Anomalous effects were found to occur at 10–15° C. owing to the production of a coloured substance "transient orange" and its decomposition. At lower temperatures this substance was found to be stable and it has been shown that the theoretical equation for the bleaching curve applied also in this range.

The results of 25 experiments showed that the extinction coefficient and the quantum efficiency are both independent of temperature and *p*H. Further arguments are put forward to support the contention that the quantum efficiency of the bleaching process is 1 or not much less than 1. On this basis a value for the molar extinction coefficient is derived which is found to be normal for this type of chromophoric grouping.

The Monaural Threshold

By J. W. Hughes

(*Communicated by T. Graham Brown, F.R.S.—Received* 26 *May* 1937)

The change of threshold intensity in one ear when a note of fixed subliminal intensity is sounded in the other has been determined by direct experiment. The results of the investigation show that for two notes in unison, the total energy required in the two ears at the "binaural threshold" is equal to the energy required in one ear at the monaural threshold, independently of the actual division of energy between the two ears. For two notes of different frequencies, the result still holds good in the form

$$\left(\frac{E_1}{E_0}\right)_R + \left(\frac{E_1}{E_0}\right)_L = 1,$$

where E_0 represents the monaural threshold intensity, and E_1 the intensity of the note present in the same ear at the binaural threshold, the subscripts R and L referring to the two ears. The bearing of the results on the theory of hearing is discussed.

The External Mechanics of the Chromosomes
VI—The Behaviour of the Centromere at Meiosis

By Margaret Upcott

(*Communicated by Sir Daniel Hall—Received* 26 *June* 1937)

The Control of Beating and of Micro-fibrillation by Means of Potassium, Calcium and Sodium in the Chick Embryo Heart
I—Calcium Fibrillation and the Control of Beating

By P. D. F. Murray

(*Communicated by Sir Henry Dale, F.R.S.—Received* 12 *July* 1937)

The principal experimental results are summarized in Table VIII. The numbers in square brackets in the summary refer to the numbers of experiments in this table.

(1) The effect of various solutions on the heart was studied [1–5]; in the majority of experiments [7–21] two or three solutions were used with the object of examining the effect of each solution on hearts previously treated with various other solutions.

(2) Hearts which had been treated with media lacking potassium were readily thrown into fibrillation by small quantities of calcium ($CaCl_2$ 0·03 %) [7, 8, 9, 10], but if small quantities of potassium (KCl 0·06 %) were present in the first solution, this protected them against the fibrillating action of small quantities of calcium in the second [5, 14, 15, 16, 17].

(3) When hearts treated with solutions lacking potassium were transferred to solutions containing calcium, but not potassium, fibrillation occurred, and was not transient [10]. But if the second solution contained both potassium and calcium, fibrillation was transient, ceasing in a few minutes [7, 8, 9].

(4) The observations recorded in a separate paper (Watchorn and Murray) show that potassium was lost from hearts when they were treated in solutions lacking potassium. There is indirect evidence that potassium was also lost in $Na+k$ [16].

(5) It is concluded (discussion) that fibrillation in the medium depends upon an abnormally high ratio between calcium at the cell surface and potassium in the cell interior, i.e. on Ca_s/K_i.

(6) Stimulation of the beat (strengthening, or restoration after loss) occurred when the hearts were transferred to a solution containing more or relatively more calcium than was present in the first solution [5, 7, 10, 14, 15, 16], unless the effect was prevented by the presence of large quantities of potassium [8, 9, 17]. If the hearts were not potassium-poor, the improved beating was maintained for long periods [14, 15, 16], but if the hearts were potassium-poor the stimulation was at once followed by stoppage of the beat. If the second solution contained no potassium the stoppage was permanent [10]; but if the second solution contained small quantities of potassium the beat subsequently returned [7].

(7) When hearts were transferred to solutions containing more, or relatively more, potassium than the first solution (but not more calcium unless potassium greatly preponderated over it), the beat was depressed or stopped [4, 8, 9, 11, 12, 13, 17, 18, 19, 21]. The inhibitory effect of potassium appeared much more rapidly if the hearts were potassium-poor than if they were not.

(8) From these and a large number of other facts it is concluded (discussion) that the influence upon beating of potassium and calcium in the medium depends upon the ratio between potassium and calcium at the cell surfaces and potassium in the cell interiors (i.e. upon Ca_s/K_i and K_s/K_i), but not upon the ratio between the two ions at the surface (i.e. Ca_s/K_s) except in so far as this affects Ca_s/K_i and K_s/K_i. Rise of the ratio K_s/K_i is inhibitory of beating, of Ca_s/K_i stimulatory, provided it does not rise too high, when it stops beating and induces fibrillation.

The Royal Society Expedition to Montserrat, B.W.I.

The Volcanic History and Petrology of Montserrat, with Observations on Mt. Pelé in Martinique

By A. G. MacGregor

(*Communicated by E. B. Bailey, F.R.S.—Received* 20 *July* 1937)

Oscillographic Study of Electron Transfer by Yellow Ferment and by Cytochromes during Muscular Contraction

By F. Urban and H. B. Peugnet

(*Communicated by D. Keilin, F.R.S.—Received* 18 *August* 1937)

An apparatus is described which records instantaneous changes in the absorption spectrum of muscle.

By means of this apparatus, it is shown that *yellow ferment* and cytochrome *c* are active during the aerobic contraction phase of a gastrocnemius muscle.

Stimulation of the Corpora Lutea of the Rat by Means of Progesterone and Testosterone

By T. McKeown and S. Zuckerman
Department of Human Anatomy, Oxford

(*Communicated by W. E. Le Gros Clark, F.R.S.—Received* 25 *August* 1937)

Normally cyclic female rats were injected with testosterone propionate or progesterone daily for from 9 to 11 days. At autopsy several recent corpora lutea were seen in the ovaries of all the experimental animals. The activity of these corpora is indicated by progestational changes in the uterus and by the production of a deciduoma in one of the rats that had been injected with testosterone. Similar endometrial changes were not induced in spayed rats that were injected with oestrone followed by testosterone or with testosterone alone. The luteinization observed in the ovaries was either a direct effect of the injections, or an indirect one due to the suppression of follicles.

Loss of Potassium from Embryonic Chick Hearts in Potassium-free Media with and without Calcium

By E. Watchorn and P. D. F. Murray

(*Communicated by Sir Henry Dale, F.R.S.—Received* 26 *August* 1937)

Factors Influencing the Effectiveness of Administered Hormones

By R. Deanesly and A. S. Parkes, F.R.S.

(*Received* 9 *September* 1937)

1. Further work has been carried out on the relation between method of administration and effectiveness of hormone preparations.

2. The response of the capon's comb and of the accessory glands of the castrated rat has been used in the study of androgenic activity and the response of the plumage of the Brown Leghorn capon in that of gynogenic activity.

3. Experiments are described on the degree of subdivision of the total dose, nature of the solvent, addition of fatty acid to the solution, esterification of the hormone, volume of medium and route of administration.

4. The influence of these different variables may be quite different for different substances, and it is concluded that no valid quantitative comparison of two compounds can be made without a careful examination of the conditions necessary for maximum effectiveness.

5. Certain of the free hormones show greatly increased effectiveness when given in dry form by the subcutaneous implantation of tablets or crystals. Recovery of the residue of the tablet from the rat after a ten-day period of experiment has made it possible to estimate the amount of hormone actually absorbed, and successive implantations of the same tablet to successive groups of rats has enabled the maximum effectiveness of a given dose to be determined.

6. The esters lose their increased effectiveness when given by intraperitoneal or intravenous injection and it is concluded that the prolongation of action by esterification is due to delay of absorption from the site of injection rather than to slow hydrolysis of the compound after absorption.

7. In general, it seems that the free hormones lose their inferiority to the esters if the method of administration ensures slow absorption, whereas the esters lose their superiority to the free hormones if absorption is rapid or instantaneous.

Studies on the Onychophora. IV

By S. M. Manton

(*Communicated by W. T. Calman, F.R.S.—Received* 15 *September* 1937)

1. The passage of spermatozoa to the ovarian lumen from the spermatophores attached to the body of the female is described.

2. Following the deposition of a spermatophore, leucocytes invade the subcutaneous region and break through the ectoderm. The cuticle of the body and the lower wall of the spermatophore are ruptured.

3. Spermatozoa swim from the spermatophore through the perforated cuticle and ectoderm and reach the vascular spaces. They pass through the haemocoel by their own activity and reach the ovary.

4. Clumps of spermatozoa force their way through the ovarian wall to reach its lumen.

5. The spermatophore wall remains attached to the cuticle, so closing the wound caused by the entry of the spermatozoa. The ectoderm regenerates and forms a new cuticle which is exposed at the next ecdysis.

6. The earliest oogonia in adult animals lie freely in the ovarian lumen among the spermatozoa. Their cytoplasm is invaded by sperm heads. They divide to form ova which absorb the sperm heads and grow.

7. Each ovum sinks into the germinal ridge epithelium. The formation of the egg follicle and ovarian egg membrane are described.

8. The ripe ovum passes through the follicle stalk back to the ovarian lumen. Here it emerges from the egg membrane. Fertilization takes place and the egg passes down the oviduct. The fertilized egg swells rapidly and a membrane is formed round it, which is probably the fertilization membrane. The oviduct secretes a second very thick membrane outside the first; only this second membrane persists during embryonic life.

9. The spermatozoa in the ovary (1) fertilize the eggs, (2) provide the early ova with nutriment necessary for their growth, and (3) may supply the animal with nourishment or other special substances.

Studies in Stimulation Phenomena in Plants due to *Bacterium solanacearum*

By B. J. Grieve

(*Communicated by A. J. Ewart, F.R.S.—Received* 21 *September* 1937)

1. A detailed study has been made of petiole-epinasty in plants invaded by the vascular parasite, *Bacterium solanacearum* E.F.S. It has been shown to be an irreversible growth reaction and has been demonstrated to occur in infected potato, tomato, castor oil and African Marigold plants.

2. The reaction has been shown to occur mainly when invasion of the petioles is unilateral or apparently unilateral. It may also occur before the invading organisms enter the petiolar bundle.

3. Petiole-epinasty is conditioned by the plant variety, its size, the point of inoculation, and by environmental conditions. A high water content in potentially reactive petioles is a necessary condition for the occurrence of the growth reaction.

4. The growth reaction has been shown to be independent of gravity.

5. Experiments designed to determine the proximate cause of petiole-epinasty showed that the bacterial secretion heteroauxin, while being produced in culture media, was not produced in sufficient amount in the invaded vessels to bring about the growth reaction. Tests to determine whether the alkaline reaction in invaded vessels, or the ammonia to which it is largely due, was a causal factor in petiole-epinasty, gave negative results. It was found, however, that the opposite condition of hyponasty could be induced in plant parts by placing them in certain acid buffer solutions. The evidence points to the conclusion that it is some toxic by-product of bacterial metabolism, as yet unidentified, which acts as the proximate cause of the reaction.

6. A possible mechanism of the growth reaction is suggested and the importance of the water factor in relation to this is discussed.

The Effect of Freezing on Bacteria

By R. B. Haines

(*Communicated by Sir Joseph Barcroft, F.R.S.—*
Received 25 *September* 1937)

1. Rapid freezing of aqueous suspensions of bacteria under defined conditions leads to death of a constant proportion of the cells, varying

from about 80 % with the most sensitive organism, *B. pyocyaneus*, to little or none with spores.

2. The temperature or rate of freezing appears to have little effect on the mortality within specified limits.

3. Storage of aqueous suspensions of bacteria in the frozen state at temperatures from −1 to −20° C. gives a most rapid rate of death near the highest temperature of storage.

4. It is shown that storage at −2° C. of a frozen solution of the native cellular proteins of *B. pyocyaneus*, extracted at low temperature, leads to rapid coagulation of one fraction of the proteins. Such flocculation is negligible at −20° C.

5. It is suggested either that two factors are responsible for the death of the bacterial cell on freezing: one unknown, but apparently not mechanical, and the other, some change leading to flocculation of the cellular proteins; or else that there is in reality one process, leading to coagulation, but with a time-lag, so that coagulation is not found on immediate freezing and thawing.

Dominance in Poultry; Feathered Feet, Rose Comb Internal Pigment and Pile

By R. A. Fisher, F.R.S.

(*Received* 27 *September* 1937)

The Suppression of Oestrus in the Rat during Pregnancy and Lactation

By T. McKeown and S. Zuckerman

From the Department of Human Anatomy, Oxford

(*Communicated by W. E. Le Gros Clark—Received* 5 *October* 1937)

Destruction of the corpora lutea of both ovaries of seven rats by means of a thermocautery at various times between the third and seventh days of pseudopregnancy led to the reappearance of oestrus within 4 days (experimental dioestrus of 7 to 11 days). The control pseudopregnancy in five unoperated animals lasted 12 to 15 days.

The corpora lutea of three pregnant animals were destroyed and the foetuses and placentae were removed on the 6th, 6th and 7th days of gestation respectively. Oestrus occurred 4, 7 and 3 days later respectively. The corpora alone were destroyed in two pregnant animals on the 4th and 5th days of gestation; oestrus reappeared on the 8th and 9th days respectively. In three control operations Caesarian section on the 6th, 7th and 7th days led to the reappearance of oestrus on the 12th, 14th and 14th days respectively.

Oestrus did not follow destruction of the corpora lutea of 12 rats which were operated on at various times between the 11th and 16th days of pregnancy. Two of these animals aborted as the result of the operation and most of the others showed partial resorption of the uterine contents. In only one was pregnancy normal.

Oestrus did not occur in four lactating animals that were left with their litters after destruction of the corpora lutea of both ovaries. Oestrus reappeared within six days in seven of eight lactating animals that were weaned soon after the same operation.

These facts show that the placentae as well as the corpora lutea have the power of suppressing oestrus, and that the progestin-threshold for the inhibition of oestrus in the second half of pregnancy is relatively low. The suppression of oestrus during lactation is probably due to the lack of follicle-stimulating hormone, but both the corpora lutea and the lactogenic hormone of the pituitary are also able to inhibit oestrus.

Progonadotropic sera

By I. W. Rowlands

(*Communicated by A. S. Parkes, F.R.S.—Received* 20 *October* 1937)

The serum of a sheep receiving a course of injections of extract of sheep pituitary did not show antigonadotropic activity, but possessed the power to augment the activity of an extract prepared from the same species on test rats.

The serum of a goat injected with pig pituitary extract augmented the action of pig, sheep and ox pituitary extracts in promoting ovarian growth in the immature rat; at the same time, this serum inhibited the ovulation-producing activity of the same extract of pig pituitary in the oestrous rabbit. The serum also inhibited the gonadotropic action on the rat ovary of extracts of horse pituitary, pregnant mare serum and human urine of pregnancy.

This augmentatory action is perhaps explicable by reference to Evans's pituitary antagonist.

The Specificity of Antigonadotropic Sera

By I. W. Rowlands

(*Communicated by A. S. Parkes, F.R.S.—Received* 20 *October* 1937)

The specificity of various antigonadotropic sera has been investigated, and an attempt has been made to classify the reactions according to "species-specificity" and "source-specificity".

Antisera were obtained to gonadotropic extracts of ox pituitary, horse pituitary, human urine of pregnancy and pregnant mare serum. All were prepared in rabbits, except an additional antiserum to urine of pregnancy extract which was prepared in a goat. The activity of the antisera was tested by their ability to inhibit the effects on immature rats and oestrous rabbits of gonadotropic extracts of horse, ox, sheep, pig and human pituitaries, human and mare pregnancy serum and human urine of pregnancy and placenta.

Complete "species-specificity" is shown by the reaction of the antisera to gonadotropic extracts of human urine of pregnancy and pregnant mare serum, whereas antisera to similar extracts of pituitary origin exhibit only incomplete "species-specificity". Complete "source-specificity", on the other hand, is only found in the reactions of the antiserum to gonadotropic extracts of pregnant mare serum.

The difficulties encountered in the interpretation of these results and their bearing on the probable occurrence in the pituitary of separate follicle-stimulating and luteinizing hormones and of a gonadotropic antagonist is discussed, and it is concluded that much more evidence is required before such specificity as is shown by antigonadotropic sera can be ascribed to immunological factors.

The Effect of Sex Hormones on Lactation in the Rat

By S. J. Folley and S. K. Kon

National Institute for Research in Dairying, University of Reading

(*Communicated by A. S. Parkes, F.R.S.—Received* 20 *October* 1937)

1. Administration of oestradiol monobenzoate (approximately 0·3 mg. per 100 g. body weight daily) to intact lactating rats causes a marked inhibition of lactation.

2. In rats ovariectomized on the day following parturition, the inhibitory effect of similar doses of oestradiol monobenzoate, while definite, was much less marked.

3. Testosterone propionate (approximately 0·4 mg. per 100 g. body weight daily) administered to lactating rats also inhibited lactation, while progesterone and androsterone in doses of approximately 0·3 mg. per 100 g. body weight daily had no effect on lactation.

4. Rats ovariectomized on the day following parturition and then given daily injections of sesame oil lactated satisfactorily.

5. The bearing of these results on the theoretical aspect of lactation is discussed.

The Genetical and Mechanical Properties of the Sex-chromosomes

IV. The Golden Hamster

By P. C. Koller

(*Institute of Animal Genetics, University of Edinburgh*)

(*Communicated by A. S. Parkes, F.R.S.—Received* 20 *October* 1937)

1. The diploid number of chromosomes in the golden hamster (*Cricetus auratus*) is 38. The chromosomes at mitotic metaphase show differences in size and shape.

2. The sex chromosomes are the largest of the complement. The *X* and *Y* chromosomes of the male are morphologically very alike and show no significant size difference. They are approximately 4·5–5 long, and have

a subterminal centromere. The ratio of the length of the two arms is about 1 : 4.

3. During meiotic prophase the sex complex is composed of two regions; one a precociously condensed, the other non-condensed or diffuse. The former is identified as the associated homologous or pairing segments of the X and Y; the latter gives rise to the differential segment of the X.

4. Two types of sex bivalent are formed with unequal frequency at meiotic metaphase; asymmetrical (81·6 %), indicating pre-reduction, and symmetrical (18·4 %) conditioning the post-reductional segregation of the differential segment.

5. The centromere is localized in the pairing segment; consequently chiasmata representing genetical crossing-over between the X and Y chromosomes, can be formed on both sides of the centromere. At metaphase the chiasma-frequency in the sex bivalent is less than in the autosomal bivalents.

6. The sex chromosomes are unpaired or fail to form chiasmata after pairing in about 8 % of the primary spermatocytes. The failure is brought about by the strong precocity of the pairing segment.

7. Analysis of the morphological structure and behaviour of the differential segment suggests that at meiotic metaphase it has minor relic and interchromatid relational coiling. Coils, which are characteristic of the pairing segment and of the autosomal bivalents, are absent.

8. It is predicted that in hamster there are great numbers of genes, which will exhibit very little sex linkage, though they are borne in the sex chromosomes. These genes are localized in the pairing segment of the X and Y.

The Geological History of the Lake Rudolf Basin

By V. E. Fuchs, M.A., Ph.D.

(*Communicated by O. T. Jones, F.R.S.—Received* 25 *October* 1937)

This is an account of the geology of an area the greater part of which has not been described. It is important because it includes the northern continuation of the Kenya Rift Valley fractures and because it extends to the Central African region of which the climatic and volcanic history is known.

The first movements which affected the region began in the late Oligocene and continued periodically into the Upper Pleistocene. Particular interest is attached to these movements for not only do they form the northern continuation of the Kenya fractures, but there is some evidence in this region that they were of a compressive nature.

The initial fracture cut off the north extension of the late Oligocene peneplain of Uganda, and upon its downthrow side a series of Miocene sediments and volcanics accumulated. The former are correlated with Gregory's Nyasan (Kamasian) deposits of the Baringo area in the south. The whole Miocene series was then folded from east to west and accompanied by faulting; these Pliocene movements gave rise to the topography of the early Pleistocene lake basin. The deposits formed in the latter were disturbed by still later movements which, accompanied by vulcanicity, completed the lake basin in its present form.

Today the lake does not occupy so large an area as it did because fluctuating but progressive desiccation has lowered its level by more than 300 ft. since Acheulian times. Everywhere its past high-water marks are defined by lake beaches containing mammalian and molluscan remains together with the stone implements of early man. An interpretation of the evidence afforded by these beaches is offered and the events within the Lake Rudolf Basin are correlated with those already known in Kenya and Uganda. Modern conditions indicate that the desiccation of the area still continues.

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

In accordance with a resolution of Council, summaries or abstracts of papers are to be published as soon as practicable. The publication of such abstracts in no way indicates that the papers have been accepted for publication in any fuller form. These abstracts will be issued for convenience with the "Proceedings of the Royal Society of London" but do not form a part of the "Proceedings".

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7 December 1937

Significance Tests for Continuous Departures from Suggested Distributions of Chance

By H. Jeffreys, F.R.S.

(*Received* 1 *November* 1937)

A method is developed for testing whether a set of observed frequencies support a uniform distribution of chance or a suggested departure from it, and is applied to linear and harmonic departures. A modification applicable to grouped data is given, and a possible way of extending the method to test departures from suggested non-uniform laws of chance is described.

The Male Sexual Stage in Salmon parr (*Salmo salar* juv.)

By J. H. Orton, J. W. Jones and G. M. King

(*Communicated by J. Gray, F.R.S.—Received* 2 *November* 1937)

A high proportion, about 40 %, of the population of two- and three-year-old salmon parr become sexually mature in October in the River Dee, Wales and Cheshire. There is virtual proof that they spawn on the immigrating adult female salmon redds before migrating to the sea as smolts. Mature and maturing male parr have been observed constituting a large proportion of the population in other rivers in Great Britain, Norway and Finland, whence it is highly probable that most or all of the males of *Salmo salar* normally attain their first sexual maturity and spawn in the river in which they are reared. The phenomenon of early sexual maturity in the salmon is comparable with late paedogenesis.

The Two-dimensional Hydrodynamical Theory of Moving Aerofoils—II

By Rosa M. Morris, B.Sc.

(*Communicated by L. N. G. Filon, F.R.S.—Received* 2 *November* 1937)

The discussion of our solution of the problem of a general cylinder moving in liquids is generalized to include the effect of a vortex sheet wake extending backwards in the liquid from a trailing edge. Assuming the wake to be of any shape and to have associated with it a total circulation which is equal and opposite to that of the main circulation round the cylinder, general formulae are derived for the potential function of the fluid motion and the resultant of the fluid pressures on the cylinder boundary.

As steps in the analysis the solution of the problem of this general cylinder at rest (i) in a uniform stream, (ii) in a stream due to an external line vortex parallel to its axis, are solved in detail.

The general formulae are then examined in their approximate form under conditions analogous to those assumed for the particular problems whose

solutions are already known, with the object (i) of determining the first order effects of camber and thickness, and (ii) of verifying the approximate theoretical results now used in practice. The result that the camber and thickness have no effect on the force (lift or drag) and only a small effect on the couple when there is acceleration, is verified but in a form which shows the precise conditions governing the degree of approximation involved.

The result for the lift arising from the circulations, however, differs from that usually given. It is shown that under the usual conditions assumed for the wake, the lift force produced is greater than that produced by the corresponding simple circulation, and that this greater lift is attained without the drag which accompanies it in the simple circulation.

On the Velocity and Temperature Distributions in the Turbulent Wake behind a Heated Body of Revolution

BY S. TOMOTIKA

(*Communicated by G. I. Taylor, F.R.S.—Received* 3 *November* 1937)

In the present paper, Taylor's modified vorticity transport theory of turbulence is applied to the calculation of the velocity and temperature distributions in the turbulent wake behind a solid of revolution which is placed in a uniform stream such that its axis of revolution is parallel to the direction of the undisturbed velocity.

In order to carry out the calculation, it is assumed, with Prandtl, that for sufficiently high Reynolds numbers and at a sufficient distance downstream there is geometrical and mechanical similarity in different sections of the wake and that the values of the mixing length at corresponding points in different sections are proportional to the breadths of the sections. Also, the isotropy in turbulence is assumed.

Assuming the mixing length to be constant over any one section, the distribution of mean velocity is first calculated and the result is compared with the results of Schlichting's and Simmons's observations. The agreement between theory and observations is not quite satisfactory.

Next, the distribution of temperature is calculated. However, the comparison of the theoretical result with observations is not made, because no measurements of the distribution of temperature in the wake behind a heated body of revolution have yet been made.

Application of the Modified Vorticity Transport Theory to the Turbulent Spreading of a Jet of Air

BY S. TOMOTIKA

(*Communicated by G. I. Taylor, F.R.S.—Received* 3 *November* 1937)

The turbulent spreading of a jet of air emerging from a small circular aperture is discussed on the basis of Taylor's modified vorticity transport theory of turbulent motion. Assuming the isotropy in turbulence, the distribution of mean axial velocity is calculated for any one section of the jet whose distance from the aperture is great in comparison with the diameter of the aperture. The calculated curve is compared with the Göttingen measurements and the fairly satisfactory agreement between theory and observations is found, as in the case of Tollmien's calculation on the basis of Prandtl's momentum transport theory.

Croonian Lecture—The Transport of Materials in Plants

BY H. H. DIXON, F.R.S.

(*Received* 5 *November* 1937)

Movement of water regarded as motion down a vapour-pressure gradient. Need of cohesion. Conditions of aqueous cohesion. Measurement of the tensile strength of sap. Demonstration of the tensile state of the water in the conducting tracts (wood, xylem) of plants. Action of adjacent living cells. "Suction pressure", rarely a lifting force, comes automatically into equilibrium with the vapour pressure in the conducting tracts at the same level. Hence observed "suction-pressure" gradient. Osmotic pressure in cell vacuoles, compared to supporting structures in the conducting capillaries. Correspondence between the osmotic pressures and the resistance experienced by the transpiration stream. Transpiring cells secretory; abstract the solvent from the solution. Transpiration into saturated spaces by means of respiratory energy. Tension in solvent; solutes in compression.

Temperature and cell permeability. Local fluctuations of temperature lead to changes of permeability and reversals in water-movement. Trans-

piration current carries solutes. Wood a channel of transport. Mason and Maskell's work; Bast (phloem) also an effective passage for solutes. Mechanism of movement. Muench's hypothesis. Thermo-electric method demonstrates simultaneous upward and downward movements.

Transport of growth substances.

Movement of virus compared to autocatalytic advance.

On the Motion of a Fluid Heated from below

By R. J. Schmidt and O. A. Saunders

(*Communicated by S. Chapman, F.R.S.—Received* 11 *November* 1937)

If a horizontal layer of fluid, initially at rest, is heated from below, instability and cellular motion set in when $\lambda\,(\equiv \alpha gc(\theta_1-\theta_2)\,d^3/k\nu)$ is about 1700. Experiments for several layer depths show that on further heating the layer becomes completely turbulent at about $\lambda = 45{,}000$. Observations by an optical method indicate that in the case of water the turbulence develops suddenly, but that in air the transition is more gradual, the first signs of turbulence occurring at about $\lambda = 5000$. The rate of heat transfer is measured at each stage, and the increase at the change from cellular to turbulent motion is found to be less than that at the change from equilibrium to cellular motion.

In the cellular motion the horizontal length of the side of a cell is found to be twice the layer depth. The vertical temperature gradients at positions on the plane midway between the boundary surface decrease to negligible values as the motion develops with increasing λ. The horizontal temperature gradients are greatest at the mid-plane.

In the turbulent motion the fluctuating temperature gradients are very small on the mid-plane. The horizontal gradients are very small on the mid-plane, but have maxima at positions between the mid-plane and either boundary surface.

The Crystal Structure of Insulin—I

By Dorothy Crowfoot

(*Communicated by R. Robinson, F.R.S.—Received* 11 *November* 1937)

X-ray crystallographic measurements show that insulin crystallises in the rhombohedral system, space group $R3$. The dimensions of the unit cell are $a = 44{\cdot}4$ A, $\alpha = 114^\circ\ 28'$ or, giving hexagonal axes, $a = 74{\cdot}8$, $c = 30{\cdot}9$ A correct to about 0·5 %. The densities of three samples of crystalline insulin have been measured in four different immersion media and show small variations between values of 1·292 and 1·314. From these and the cell dimensions the unit cell molecular weight may be calculated as most probably 39,700 (maximum 40,800, minimum 38,200). The crystals contain water which they lose when dried at 104° in a vacuum and this has been estimated at 5·35 %. The molecular weight of protein in the unit cell is hence calculated as 37,600 (33,900–36,000), which is of the order of magnitude though rather less than the molecular weight of insulin found by Svedberg. There is however no crystallographic evidence that this weight constitutes a single molecule.

The intensities of all the X-ray reflexions that could be observed from single crystals of insulin have been estimated visually and are recorded as terms proportional to F_2. From these terms a series of Patterson-Harker Fourier synthesis have been formed which show the distribution of the main interatomic distances within the insulin molecule. The patterns correspond to a fairly large scale structure and as such cannot give information on the placing of individual atoms or as to whether the trigonal symmetry shown is necessarily carried to atomic dimensions. The most important interatomic distances that appear are at 10 and 22 A roughly parallel to the basal plane. It is suggested that these are related to the characteristic X-ray spacings of many proteins both globular and fibrous, and the virus proteins.

The Effect on the Growth of *Lemna minor* of Alternating Periods of Light and Darkness of Equal Length

By H. Dickson

(*Communicated by V. H. Blackman, F.R.S.—Received* 12 *November* 1937)

Apparatus has been designed to enable *Lemna* to be grown in controlled conditions and under various equal alternating periods of light and darkness ranging from alternations of 5 sec. to 12 hr.

Sixteen experiments are recorded in which the rate of frond increase and the dry weight per frond were determined continuously under several different lighting conditions for periods of up to 9 weeks duration.

It has been shown that the relative rate of increase of dry weight is at a maximum in continuous light. Under 12 hr. and 5 min. alternating periods the rate was lower, but as the light intensity was the same in all cases, and the plants in continuous light consequently received twice the amount of those in the alternating periods, the efficiency was much higher in alternating light than under continuous illumination. The rate was lowest at 1 min. alternations but with 5 sec. alternating periods it was much higher, being even significantly higher than with 12 hr. illumination.

Curves of the relative rate of increase in frond number and of the average weight per frond were similar to those for rate of dry-weight increase, except that the rate in 5 sec. alternating periods was not significantly different from that in 12 hourly alternations.

Portsmouth (1937) attributed the small growth rate of cucumber plants in one minute alternating periods as probably due in part at least to closure of the stomata. Observation shows that the stomata of *Lemna* are non-functional, so that in view of the fact that decrease in growth-rate at 1 min. alternating periods is as marked in *Lemna* as it is in cucumber plants it appears more likely that stomatal closure is a secondary effect rather than the cause of the drop in growth rate.

The appearance of the plants in 1 min. alternating periods is very different from that under other conditions of illumination. The fronds are small in size and not glossy, and lie at all angles to one another. This change in morphology takes place comparatively suddenly.

An Analysis of the "Pleiotropic" Effects of a New Lethal Mutation in the Rat (*Mus norvegicus*)

By G. Grüneberg

(*Communicated by J. B. S. Haldane, F.R.S.—Received* 12 *November* 1937)

"Pleiotropism" of genes can theoretically arise in three different ways. Two effects *a* and *b* may either be produced by the gene directly by means of two different mechanisms; or they may be produced by the gene directly by means of the same mechanism; or the gene may produce only one effect *a*, which in turn conditions *b* (indirect gene action). In the latter two cases, unity of gene action is maintained (spurious pleiotropism), whereas only the first case would constitute genuine pleiotropism, the gene producing manifold effects in different ways.

The analysis of the manifold effects of a new recessive lethal factor in the rat, which is linked to pink-eye dilution, shows that the most fundamental disturbance so far discovered is an anomaly of the cartilage; this single mechanism affects several structures of the body in a similar way. Hyperplasia of the ribs and of the cartilage rings of the trachea thus produced leads secondarily to the development of an emphysema of the lungs, and the animals ultimately die from more or less remote consequences of this emphysema.

Unity of gene action is therefore maintained in this case, which, despite the complex ramifications of the effects, is only an example of spurious pleiotropism. This seems also to apply to the few other cases which are fully analysed. The existence of genuine pleiotropism has never been conclusively proved. As unity of gene action is the simpler conception, and as it has been shown to explain fully analysed cases, it may be adopted as a general principle unless and until the occurrence of genuine pleiotropism has been conclusively demonstrated.

Resonance in Crystal Beams of Sodium-Ammonium Seignette Salt

By W. Mandell

(*Communicated by O. W. Richardson, F.R.S.—Received* 13 *November* 1937)

Experiments are made with longitudinal oscillations in crystal beams of sodium-ammonium seignette salt. For a square beam a theory is deduced for the interaction of two longitudinal oscillations at right angles. These are considered as analogous to two coupled electrical circuits. If the natural frequencies in the two directions agree to within a small number of cycles per second, the theory requires that resonance shall occur with the production of two new fundamental frequencies. This result is demonstrated experimentally.

Experiments are also made to determine the change in wave-length with change in breadth and thickness. Theoretical calculations are made of the lateral contractions along the breadth and along the thickness, accompanying extensions along the length. The effect of thickness on the wave length is found to be very small, in spite of the fact that the lateral contractions are large in two types of beams used. A change of breadth may change the wave-length by 20 %. The experimental results for two types of beam agree closely with an empirical formula, while one type of beam behaves in an anomalous manner.

On the Mechanism of the Decomposition of Hydrogen Peroxide by Catalase

By D. Keilin, F.R.S. and E. F. Hartree, Ph.D.
The Molteno Institute, University of Cambridge

(*Received* 15 *November* 1937)

1—A new explanation of the mechanism of the decomposition of H_2O_2 by catalase is proposed. According to this explanation the reaction is brought about by the successive reduction of the catalase iron by H_2O_2 and its

reoxidation by molecular oxygen; the reaction proceeding according to the following equation:

$$\begin{array}{c} 4Fe^{\cdot\cdot\cdot} + 2H_2O_2 = 4Fe^{\cdot\cdot} + 4H^{\cdot} + 2O_2 \\ 4Fe^{\cdot\cdot} + 4H^{\cdot} + O_2 = 4Fe^{\cdot\cdot\cdot} + 2H_2O \\ \hline 2H_2O_2 = 2H_2O + O_2 \end{array}$$

2—This explanation is supported by the following experiments:

a—It has been shown manometrically that the decomposition of H_2O_2 by catalase does not proceed in absence of molecular oxygen.

b—It has been shown spectroscopically with azide- and hydroxylamine-catalase that H_2O_2 is the only known substance capable of reducing catalase iron, which can then be readily reoxidised by molecular oxygen.

3—The inhibitors of catalase can be separated into two groups, those like KCN, H_2S, C_2H_5OOH which inhibit the reduction of catalase iron by H_2O_2 and those like azide, hydroxylamine and hydrazine which inhibit the reoxidation of the reduced catalase by molecular oxygen (Keilin and Hartree, 1936).

4—The decomposition of H_2O_2 by pure catalase preparations is not inhibited by CO in presence of even a very small concentration of oxygen, which shows that the reduced catalase has a much greater affinity for oxygen than for CO.

5—Some catalase preparations have been obtained which show a more or less marked light sensitive inhibition by CO. Pure catalase preparations become sensitive to CO in presence of a very small concentration of azide, cysteine or glutathione, substances which apparently inhibit the reoxidation of reduced catalase.

Instability of Fluids Heated from below

By Krishna Chandra, M.Sc.

(*Communicated by Sir Gilbert Walker, F.R.S. Received* 20 *May* 1936—*Revised* 12 *November* 1937)

A layer of air in a shallow chamber was made unstable by heating from below, and the motion which ensued, made visible by smoke, was studied for a range of depths of the chamber, and for a range of differences of temperature between top and bottom of the chamber. It was found that, unless the excess of temperature at the bottom exceeded a certain critical value, no motion took place. In depths of 10 mm. or more, the motion observed when the critical value was exceeded was in the form of polygonal prismatic cells, with descending motion at their centres, and the critical difference of temperature necessary to produce motion was in good agreement with the theoretical formula given by Jeffreys. In depths of less than 10 mm., the motion observed when the critical difference of temperature was first exceeded was of a different character, the ill-defined smoke structures having ascending air at their centres, and the critical difference of temperature was less than that given by Jeffreys' theory. It was found that with still higher differences of temperature the motion in depths of 7–10 mm. took the form of polygonal cells, the change in type of motion occurring at critical differences of temperature which were in agreement with the theoretical expression given by Jeffreys.

When the air in the chamber was subjected to a shear by motion of the top bounding plate, the smoke patterns in the chamber, when the initial conditions were such as to give polygonal cells in the absence of shear, were in the form of transverse rolls, distorted polygonal cells, or longitudinal pairs of roll vortices, according to the rate of shear and the difference of temperature in the chamber. If the motion in the absence of shear were of the other type, the effect of shear was always to give spindle shaped structures, whose length was increased by increase of the rate of shear.

Studies on the Origin of the Larval Kidneys of the Mollusca

I—Pulmonata Stylommatophora

By W. Fernando

(*Communicated by E. W. MacBride, F.R.S.—Received* 16 *November* 1937)

From the mesodermal bands differentiate the rudiments of the larval kidneys in *Achatina*, *Glessula*, and *Opeas*.

In *Achatina* this mesodermal rudiment isolates from the remainder of the mesoderm and develops a lumen inside. An ectodermal invagination later unites with the mesoderm and a tubular larval kidney results, opening at one end to the exterior. In *Glessula* the isolation of the mesodermal rudiment is less marked than in *Achatina*, but subsequent development is the same. In *Opeas* the mesodermal rudiment of the larval kidney is differentiated at a very early stage while still in contact with the rest of the mesodermal band. The ectodermal invagination also develops very early but the union of the two portions takes place at a later stage.

The larval kidneys of the Stylommatophora are for the greater part mesodermal, only a small portion with the external aperture being formed of ectoderm.

The Reflection Coefficients of Ionospheric Regions

By E. V. Appleton, F.R.S. and J. H. Piddington

(*Received* 17 *November* 1937)

Experiments on the radio sounding of the ionosphere are described which give no support to the supposed existence of permanent highly reflecting regions at heights of 10 km. It is considered possible that the echoes of short delay noted by previous workers are due to scattering from patches of ionisation of very low effective reflexion coefficient. It is found that, after the normally recognized regions of the ionosphere, the radio reflecting centres in the atmosphere next in order of importance as regards wireless echo production are transitory bursts of ionisation which occur at equivalent heights of from 80 to 160 km. Such bursts of ionisation are due to some cosmic agency which is usually effective throughout the whole of the day and night.

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

In accordance with a resolution of Council, summaries or abstracts of papers are to be published as soon as practicable. The publication of such abstracts in no way indicates that the papers have been accepted for publication in any fuller form. These abstracts will be issued for convenience with the "Proceedings of the Royal Society of London" but do not form a part of the "Proceedings".

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22 December 1937

On the Cascade Theory of Electronic Showers

By Dr L. Landau and Dr G. Rumer

(*Communicated by P. Kapitza, F.R.S.—Received* 18 *November* 1937)

1. Starting from the physical idea proposed by Bhabha and Heitler, and Carlson and Oppenheimer on the cascade production of showers, a more rigorous treatment of the problem is given (§§ 1–5).

2. A table is given for the calculation of the number of the shower particles as function of penetrated depth for any given initial energy of the creating particle and also of the energy distribution for shower particles at a given depth (§ 6).

3. The showers possess a definite range (§ 7).

4. The behaviour of shower passing from air to a medium with a different atomic number is studied (§ 9).

5. The consequences arising from the hypothesis of the new "semi-heavy" particles are discussed.

The Crystal Structure of Pentaerythritol Tetracetate

By Thomas H. Goodwin and Rowland Hardy

(*Communicated by W. L. Bragg, F.R.S.—Received* 18 *November* 1937)

The crystal structure of pentaerythritol tetracetate has been determined by the method of X-ray analysis. This is the first ester to be examined in detail and the results show:

(*a*) that the valencies of the central carbon atom are inclined to each other at the tetrahedral angle ($109\frac{1}{2}^\circ$);

(*b*) that the acetate group $—O.CO.CH_3$ has a planar configuration;

(*c*) that the valency angle of oxygen $\diagdown O \diagup$ is $109\frac{1}{2}^\circ \pm 3^\circ$ in this compound;

(*d*) that the length of the $C=O$ bond in an ester is $1{\cdot}33 \pm 0{\cdot}03$ A.

The first two of these conclusions are in agreement with stereo-chemical theory. In view of the assertions of Gerstäcker, Möller and Reis (1928) it seems desirable to call attention to (*a*) which indicates that the central carbon atom has a tetrahedral and not a pyramidal distribution of valencies.

The co-ordinates of all the atoms in the unit cell have been determined by means of two- and three-dimensional Fourier syntheses. It is believed that this is the first compound for which three-dimensional syntheses (sections through the unit cell at different heights or along special lines) have been made.

Critical Phenomena in Gases

II—Vapour Pressures and Boiling Points

By J. E. Lennard-Jones, F.R.S. and A. F. Devonshire

(*Received* 24 *November* 1937)

This paper extends to liquids the theory which was given recently by the authors for the equation of state of a gas at high densities. A direct calculation is made of boiling points in terms of interatomic forces and the numerical results for the inert gases are in satisfactory agreement with the observed values. A theoretical interpretation is given of Trouton's empirical rule connecting the heat of vaporization with the boiling temperature and also of Hildebrand's modified form of it. Calculations are made of the heats of vaporization of the inert gases.

The Electronic Structure of some Polyenes and Aromatic Molecules

IV—The Nature of the Links of certain Free Radicals

By C. A. Coulsdon

(*Communicated by J. E. Lennard-Jones, F.R.S.—*
Received 24 *November* 1937)

General formulae are given for the lengths of the links of free radicals and molecules of chain form $C_{2n+1}H_{2n+3}$. It is shown that in these chains the effect of resonance is to remove some of the characteristic properties of alternate single and double bonds. Calculations have been made of the energies of these radicals in particular cases, and the effect of resonance has been estimated.

The Electronic Structure of some Polyenes and Aromatic Molecules

V—A Comparison of Molecular Orbital and Valence Bond Methods

By G. W. Wheland

(*Communicated by J. E. Lennard-Jones, F.R.S.—*
Received 24 *November* 1937)

The valence-bond and the molecular orbital treatments of cyclobutadiene disagree in two respects. The first treatment predicts that the resonance energy should be about $-1{\cdot}5$ e.v. and that the ground state should be a singlet; the second, on the other hand, predicts that the resonance energy should be zero and that the ground state should be a

triplet. The first of these discrepancies is found to be a result of the fact that the molecular orbital treatment neglects the resonance between the two Kekulé-like structures. The second discrepancy is found to be due to the effect of ionic structures, which are not taken into account in the valence-bond treatment, but which are made important in the molecular orbital treatment. The resonance among these ionic structures is of such a nature as to stabilize the triplet considerably more than the singlet state. A similar discussion of benzene shows that the ionic structures contribute to the stability of the singlet ground state in essentially the same way as to the lowest triplet state of cyclobutadiene.

The Electronic Structure of some Polyenes and Aromatic Molecules

VI—Phenylethylene, Stilbene, Tolane and the Phenyl Methyl Radical

By W. G. Penney and G. J. Kynch

(*Communicated by J. E. Lennard-Jones, F.R.S.—*
Received 24 *November* 1937)

Calculations are made to find the internuclear distances in molecules containing the system $C{=}C{-}C_6H_5$. The length obtained for the double bond is the same as that in ethylene; the ring distances are the same as those in benzene; but the "single" bond has the peculiarly short length 1·44 A. Similarly, in the system $C{\equiv}C{-}C_6H_5$, the triple bond has a normal length 1·20 A, the ring distances are the same as in benzene, but the "single" bond has a length 1·41 A. Measurements by Robertson and Woodward on stilbene and tolane agree exactly with these results.

The Rotational Energy Levels of a Diatomic Molecule in a Tetrahedral Field

By H. M. Cundy, Trinity College, Cambridge

(*Communicated by J. E. Lennard-Jones, F.R.S.—*
Received 24 *November* 1937)

It is shown by general principles of the theory of groups how the Schrödinger equation may be solved for a diatomic molecule, when the potential energy varies with the angular coordinates in such a manner that it has the symmetry of the regular tetrahedron. Infinite series for the wave-functions are given, and the energy levels and corresponding wave-functions evaluated for a particularly simple potential-energy function of the given type. Three methods of obtaining these levels are described, two of which are asymptotic approximations, and the third involves the use of the Mallock electric calculating machine. A good agreement is obtained between the three, enabling a plot to be made of the energy levels over wide ranges of magnitude of the potential energy term.

The Potato Virus 'X'; its Strains and Reactions

By R. N. Salaman, F.R.S.

(*Received* 27 *November* 1937)

The X virus is a term which should be applied to a group of strains rather than to a single specific pathogen with fixed and constant reactions. It is shown that at least six strains are to be found distributed amongst the diseased potato plants of this country, and that most of them are represented in Germany, Canada and the U.S.A.

In the open field a potato plant is usually infected by two or more strains simultaneously, but in the case of the milder X^H and the more virulent X^N pure single strain infections do occur.

The strains range in respect to their virulence, as judged by their behaviour in tobacco and Datura from the creation of a symptomless carrier

to an intensely virulent necrosis endangering the continued existence of the host. The reaction of the strains is described on a large range of plants.

The strain X^N is shown to be the pathogen responsible for a distinctive morbid condition in the potato, here called "interveinal necrosis". The disease and the reactions of the strain are described for the first time.

Infection by any of the strains brings about the formation of inclusion bodies within the host tissues. Such inclusions do not vary in character or frequency in relation to the virulence of the strain. There is a certain variety in their appearance which may be recognized, whichever strain is infecting the host.

All the strains are made up of similar sized particles and possess certain identical serological reactions.

From plants infected by each strain the same type of nucleoprotein has been recovered, which in solution exhibits anisotropy of flow.

The physical properties of the strains are alike except for the degree of dilution which they will withstand.

The clinical results obtained by mixture of pure strains has been studied, evidence is given that a tenfold excess of a weak over a strong may lead to the clinical masking of the latter in the host plant, a lesser relation of strong to weak has the opposite result.

The double reactions that ensue from mixtures of the different X strains with the Y virus, the A virus and tobacco mosaic respectively, conform to the actual differences in virulence of the different strains themselves.

Conversion of one strain to another has been studied both in material which is relatively pure to strain as well as in that which we have good reason to believe is in reality pure. In both conversions have taken place. Conversions have been effected by passage through certain unrelated plants.

All conversions, whether purposely designed or not, have been from the more to the less virulent strain.

The nature of these conversions is discussed and the conclusion reached that they should be regarded as true mutations.

The phenomenon of acquired immunity as exhibited by the potato and other solanaceous plants in respect to the X virus is described as well as its occurrence in nature, and the possible relation of the same to the occurrence of certain types of carriers.

X-ray analysis of the Dibenzyl Series
V—Tolane and the Triple Bond

BY J. MONTEATH ROBERTSON, M.A., D.SC. AND I. WOODWARD, M.A.

(*Communicated by Sir William Bragg, O.M., F.R.S.—*
Received 27 *November* 1937)

The crystal dimensions of tolane are very similar to those of stilbene, and this fact has been used in making a very direct X-ray analysis of the tolane structure, without the use of any model based on the chemistry of the compound. The two central atoms linked by the triple bond are found to be collinear with the end atoms of the benzene rings to which they are joined. The carbon-carbon triple bond distance is 1·19 A, and for the "single" bond connecting these central atoms to the benzene ring, the unusually small distance of 1·40 A is obtained. These results are discussed in relation to acetylene and other compounds.

As in the stilbene structure, there are four molecules in the unit cell, and two of these make independent contributions to the asymmetric unit of the crystal. The orientations of these two molecules are deduced, and the co-ordinates of the atoms are given. The minimum intermolecular approach distances are between 3·5 and 3·6 A for the carbon atoms.

Further Studies on the Relationship between *Hyoscyamus* Virus 3 and the Aphis *Myzus persicae* (Sulz.) with special reference to the Effects of Fasting

BY M. A. WATSON

(*Communicated by Sir John Russell, F.R.S.—*
Received 29 *November* 1937)

The aphides (*Myzus persicae*) are much more likely to transmit *Hyoscyamus* virus 3 if they are made to fast immediately before feeding on the source of infection. Their efficiency as vectors increases rapidly during the first hour of fasting, but the rate of increase is much slower during the following 12 hours.

The increase in efficiency induced by such preliminary fasting decreases

as the infection feeding period is increased, and after one hour's feeding on the infected plants preliminary fasted aphides give no more infection than do continuously fed aphides.

Infectivity is lost by the aphides after the cessation of infection feeding, and the rate of loss is probably more rapid than the rate at which the virus deteriorates *in vitro*. This loss of infectivity is complete in 1 hour if the aphides do not fast before infection feeding, or if they are fed on an intermediate healthy plant between the infection feeding and the infection trial; but if they fast both before and after the infection feeding, the relative rate of loss of infectivity is slower, and a few insects may remain infective for many hours.

The infectivity of an aphid is greater after two or more successive infection feedings of equal length, unless the treatment given between the feedings is such that all the infectivity is lost. The infectivity actually acquired at the second or third infection feeding is, however, less than that acquired at the first, because the feeding activity of the aphid is decreased by the experimental conditions.

The effect of preliminary fasting appears to be independent of feeding activity or "appetite", and is very much more important.

It is possible to explain these results if it be assumed that, during the process of transmission from one plant to another in the recently fed aphid, the virus comes into contact with some substance which partially or wholly destroys its infectivity. The possible nature of this substance is discussed.

Tides in Oceans bounded by Meridians

III—Ocean bounded by Complete Meridian: Semidiurnal Tides

By A. T. Doodson, F.R.S.

(*Received* 29 *November* 1937)

The first two parts of this series of memoirs have dealt with the general equations for the motion in an ocean bounded by a complete meridian (Proudman 1936) and with their application to the diurnal tide (Doodson 1936). This present memoir is concerned with the semidiurnal tide (K_2).

The investigation, as in Part II, is dependent upon the formulation and numerical solution of the equations from the use of 63 co-ordinates or variables, and the solution has been effected and is illustrated for 20 values of the depth of the ocean, from very large to medium depths. Though a part of the work of solution is common to all cases, in effect 20 sets of equations, each set having 63 equations, have been solved.

The results are of exceedingly great interest in connexion with tidal theory. The genesis and development of amphidromic systems, as the depth is changed, can be fully traced. The possible systems of cotidal lines are of great variety, and the type of amphidromic system changes so rapidly with the depth that a single illustration of the tides for a particular depth is of doubtful value for comparison with an actual ocean but it is hoped that the complete series of illustrations will provide material for the derivation of principles which can be applied to terrestrial oceans.

Thermal Conduction in Hydrogen-deuterium Mixtures

By C. T. Archer, A.R.C.S., M.Sc., Senior Lecturer in Physics,
Royal College of Science, London, S.W. 7

(*Communicated by A. O. Rankine, F.R.S.—Received* 30 *November* 1937)

The paper describes an experimental investigation of thermal conduction in hydrogen, deuterium, and hydrogen-deuterium mixtures of varying concentration. The hot wire method, used by the author and others previously in similar work, was adopted to determine the thermal conductivity at 0° C. of hydrogen, deuterium, and of each mixture, and also the accommodation coefficients of hydrogen and deuterium relative to a platinum surface at 0° C.

The hydrogen was prepared from distilled water, the deuterium from deuterium oxide of 99·95 % guaranteed concentration, and the gaseous mixtures from mixtures of the water and deuterium oxide, by passing the vapour over magnesium heated to about 500° C. in an electric furnace. Deuterium was also prepared from the oxide by reaction with metallic sodium in vacuo. The percentage concentrations of the gaseous mixtures were obtained from observations of the densities of the liquid mixtures, using a flotation method.

The values obtained of the thermal conductivity at 0° C. of hydrogen and deuterium were $0{\cdot}000418_2$ and $0{\cdot}000308_0$ cal. cm.$^{-1}$ sec.$^{-1}$ deg.$^{-1}$ C., and of the accommodation coefficient $0{\cdot}29_6$ and $0{\cdot}37_6$ respectively. The values of the thermal conductivities of all the gases together with the percentage concentrations are shown in a Table, and also represented by means of a graph.

Antiplasmodial Action and Chemical Constitution

I—Cinchona Alkaloïdal Derivatives and Allied Substances

By A. Cohen and Harold King, F.R.S.

(*Received* 30 *November* 1937)

Experimental bird malaria is utilized for following the effect of changes of structure in the cinchona alkaloids on the therapeutic activity. It is shown that although antiplasmodial action is not very sensitive to stereochemical changes, it is sensitive to changes of structure. Thus there is apparent loss of activity when the central —CHOH— group is modified in any way. Thus its conversion into —CHCl—, —CH_2— or —CH= leads in almost all cases to an increase in toxicity and loss of antiplasmodial action. The preparation and properties of a series of allied substances is also described.

Antiplasmodial Action and Chemical Constitution

II—Some Simple Synthetic Analogues of Quinine and Cinchonine

By A. D. Ainley and Harold King, F.R.S.

(*Received* 30 *November* 1937)

Quinicine (quinotoxine), the ketonic isomeride of quinine, is inactive in bird-malaria. Reduction to dihydroquinicinol (2 diastereoisomerides) with re-formation of a carbinol group does not restore activity; neither is activity found in α-*N*-methyldihydroquinicinol. Consideration of these results led the authors to the synthesis of 4-quinolyl-α-piperidylcarbinol and 4-(6-methoxyquinolyl)-α-piperidylcarbinol and their *N*-methyl, *N*-propyl, *N*-allyl, *N*-butyl and *N*-crotyl derivatives. The only compounds

obtained showing antiplasmodial activity were 4-(6-methoxyquinolyl)-α-piperidylcarbinol and its diastereoisomeride *iso*-4-(6-methoxyquinolyl)-α-piperidylcarbinol. These two substances appear to be the first simple substances built on the pattern of the cinchona alkaloid molecule to show antiplasmodial activity.

Propagation of Wave-packets Incident Obliquely upon a Stratified Doubly Refracting Ionosphere

By K. G. Booker, Ph.D.

(*Communicated by E. V. Appleton, F.R.S.—Received* 1 *December* 1937)

A study is made of the propagation of wave-packets incident obliquely upon a slowly varying plane-stratified doubly refracting medium with a view to applying the results to the ionosphere. The magneto-ionic theory in the form given by Appleton is only suitable for investigating vertical propagation in the ionosphere. The theory is generalized so as to be capable of describing oblique propagation of a magneto-ionic component through the ionosphere without using a refractive index which depends in a complicated way upon an unknown angle of refraction. The fundamental formula of the oblique-incidence magneto-ionic theory is an algebraic quartic equation for a quantity q which depends upon the prescribed angle of incidence and which at vertical incidence becomes identical with the well-known refractive index. The four roots of the quartic equation for q correspond to the upgoing ordinary wave, the upgoing extraordinary wave, the downcoming ordinary wave and the downcoming extraordinary wave. The level in the ionosphere where the individual wave-crests are moving horizontally across a characteristically polarized wave-packet is given by the condition $q = 0$, which is equivalent to putting the angle of refraction equal to $x/2$ in Snell's Law. But the level of reflexion of the magneto-ionic component is the level where the wave-packet *as a whole* is travelling horizontally and is given by the condition that the root of the quartic equation for q corresponding to the upgoing magneto-ionic component should be equal to a root corresponding to a downcoming magneto-ionic component.

The detailed application of these results to the ionosphere depends on our

ability to solve the fundamental quartic equation for q, and the only practical way of achieving this in general is to resort to one of the standard numerical methods. Special cases worked out in this way show that the critical electron density required for reflexion of a magneto-ionic component can easily be as much as 25 % in excess of the erroneous value which would be calculated by putting the angle of refraction equal to $\pi/2$ in Snell's Law.

The analytical solution of the quartic equation for q is sufficiently simple to be of practical value in the case of east-west transmission (including vertical incidence) and in the case of propagation in equatorial regions. Owing to the symmetrical influence of the earth's magnetic field upon the propagation of the upgoing and downcoming waves in these special cases it is legitimate to calculate the critical electron densities of the magneto-ionic components from the condition ($q=0$) that the direction of phase-propagation is horizontal. Representative curves are drawn.

An Experimental Determination of the Spectrum of Turbulence

By L. F. G. Simmons, M.A. and C. Salter, M.A.

With an Appendix: Method of Deducing $F(n)$ from the Measurements

(*Communicated by G. I. Taylor, F.R.S.—Received* 1 *December* 1937)

The time variation of velocity at a fixed point in a turbulent air stream is analysed into a spectrum. The method adopted involves the use of the ordinary hot-wire technique to produce changes of potential in a Wheatstone bridge circuit, which are magnified by a value amplifier. The fluctuating voltage drop generated is then applied, in turn, to electrical filters having different cut-off frequencies. In each case the output current is measured, with and without each filter in circuit, by means of a thermal milliammeter which indicates the mean value of the square of the current supplied to it. From the ratios of the readings taken with and without each filter, the spectrum curve is calculated by a method described in the Appendix.

All measurements were made in a wind tunnel, at a point in the air stream where the turbulence created by a grid of regular mesh was known to be isotropic: the wind speeds used were 15, 20, 25, 30 and 35 ft./sec.

The Spectrum of Turbulence

By G. I. Taylor, F.R.S.

(*Received* 1 *December* 1937)

It is shown that a definite connexion exists between the spectrum of the time variation in wind at a fixed point in a wind stream and the curve correlation between the wind variations at two fixed points. The spectrum curve and the correlation curve are, in fact, Fourier transforms of one another.

As an example of the use of this relationship the spectrum of turbulence in an American wind tunnel was calculated from measurements of correlation by Dryden. In some further experiments Dryden modified this spectrum by inserting a filter circuit and then measured the correlation with this filter in circuit. The modified spectrum is here calculated from the filter characteristics and the Fourier transform theorem is used to calculate the modified correlation curve. The agreement with Dryden's measurements is very good indeed.

The paper ends with some remarks on the bearing of the spectrum measurements on the theory of dissipation of energy in turbulent flow.

The Alkaline Permanganate Oxidation of Organic Substances selected for their bearing on the Chemical Constitution of Coal

By R. B. Randall, Ph.D., M. Benger, Ph.D. and
C. M. Groocock, Ph.D.

(*Communicated by Professor W. A. Bone, F.R.S.—*
Received 3 *December* 1937)

The paper describes the results of systematic qualitative and quantitative investigations of some sixty organic substances, namely, (i) carbohydrates and aliphatic carboxylic acids, (ii) aromatic hydrocarbons, (iii) aromatic substances containing $=CO$ groups, (iv) aromatic carboxylic acids and ethers, and (v) heterocyclic compounds, selected for their bearing on the coal-constitutional problem, and discusses the principal conclusions to be drawn therefrom. Details are also given of the experimental methods employed.

Indicators of Salt Accumulation by Plants; the Absorption of Rubidium Bromide by Potato Discs

By F. C. Steward and J. A. Harrison

(*Communicated by V. H. Blackman, F.R.S.—Received* 6 *December* 1937)

The paper describes the results of a quantitative investigation of the absorption of rubidium and bromide from dilute solution by discs of potato tissue. The results contribute to knowledge of the absorption process in plants.

An accurate spectrographic method for the determination of rubidium in plant extracts was devised for this investigation. The method, which is applicable to other biological problems, is described in detail.

The ions rubidium and bromide were used as sensitive indicators of the absorption of cation and anion respectively because they are not normally present in potato tissue, except in negligible amounts, they are both readily absorbed and can be estimated accurately. The experiments which were carried out under rigorously controlled conditions by a technique which has been described elsewhere, concern the effects of oxygen concentration, time and the specific surface of the discs on the absorption of rubidium and bromide.

The results establish the relationship between the metabolism of the cells and the absorption of both bromide and rubidium. The absorption of rubidium is, however, of two kinds, one which is determined by the metabolism of the tissue and the other which is independent of vital activity and may be observed in dead discs. The two types of absorption may be distinguished by their relations to the variables oxygen concentration, time and temperature. The maintained absorption of rubidium and of bromide which is dependent upon vital activity is confined to a surface layer of active cells in the discs whereas that part of the absorption of rubidium which is due merely to a property of the tissue substances occurs throughout the whole of the disc.

Experiments upon the effects of concentration upon absorption, carried out on both living and dead discs, reveal the general nature of the two absorption mechanisms and lead to a new outlook upon the problem of the relative uptake of anion and cation in plants.

The Action of Ultra-violet Radiation on Proteins, I, II

By J. S. MITCHELL

(*Communicated by E. K. Rideal, F.R.S.—Received* 8 *December* 1937)

1. A brief review is given of the photochemistry of proteins with especial reference to the process of oxidative photolysis.

2. The photochemical significance of the ultraviolet absorption spectra of proteins is discussed quantitatively, in relation to the hydrolytic analyses. Measurements of the absorption spectra of certain proteins—ovalbumin, gliadin and insulin—and of the tyrosine derivatives—glycyl-tyrosine and *N*-stearyl-tyrosine ethyl ester—are given. For the absorption of radiation of wavelengths 3150–2350 A the only residues of importance, in typical proteins, are those derived from the "aromatic" amino acids—tyrosine, phenylalanine and tryptophane—and also cystine and arginine—further, 85–100 % of the total light absorption is due to the three "aromatic" amino acids. Thus the proteins must be regarded as macromolecular polycondensation products in which only a small fraction of the total number of repeating units possess chromophoric groups and can take part in primary photochemical processes.

At *p*H 2 and 7·2 the assumption of linear additivity of the absorption coefficients of the individual amino acids of proteins is a poor approximation, but at *p*H 12 at the wavelength of the absorption maximum, it is possible to calculate the molecular extinction coefficient of insulin with an error of less than 3 %.

3. The photochemical decomposition, in aqueous solution, of ovalbumin, insulin, glycyl-tyrosine and l-dihydroxyphenylalanine ("dopa") by the approximately monochromatic radiation, of wavelength 2537 A, given by a mercury-neon discharge tube, has been examined chemically and by observation of the changes in the ultraviolet absorption spectra.

4. Ovalbumin, in solution in the presence of oxygen, undergoes complete photo-oxidative decomposition with production of proteoses as end-products, and disappearance of the reactions characteristic of tyrosine, tryptophane and cystine; the changes in the absorption spectrum emphasise the importance of the aromatic groups.

It appears from the present and previous work that, in general, the end-products of unsensitized photolysis of proteins with light of wavelengths longer than 2350 A are to a large extent fragments of high molecular weight and not the constituent units, thus suggesting selective photochemical attack at localized sites in the molecule.

5. A detailed examination has been made of the photochemistry of glycyltyrosine in solution, to test the hypothesis that the photo-oxidative decomposition of proteins with light of wavelengths longer than 2350 A is due to molecular fragmentation at the sites of attachment of the aromatic chromophors. Ancillary studies of dopa and indol were made. As a result of light absorption in the aromatic nucleus, glycyl-tyrosine undergoes a series of photo-oxidative transformations—involving the formation of dihydroxyphenyl compounds, probably orthoquinones, and indol derivatives—which result in fragmentation of the peptide linkage with production of glycine; this last step is probably one of photochemical hydrolysis.

6. This mechanism of indirect oxidative photolysis of the peptide linkage adjacent to the aromatic chromophor appears applicable to proteins. The changes in the absorption spectrum on ultraviolet irradiation in presence of oxygen have been compared, in the case of insulin—where the only important chromophors are those derived from tyrosine—and of glycyl-tyrosine; it must be concluded that there is no fundamental difference between the photo-oxidative transformations of the tyrosine chromophors in the two cases.

New Bands Ending on the $1s\sigma 2p\sigma\,{}^1\Sigma_4$ State of H_2

By O. W. Richardson, F.R.S.

(*Received* 8 *December* 1937)

This paper describes two new band systems which go down to $1s\sigma\,2p\sigma\,{}^1\Sigma u$, the state on which the strongest hitherto known systems in the visible end. The systems extend from the yellow to the photographic infra-red limit (8500 A) and probably beyond. The upper states are both ${}^1\Sigma g$ states. They are very close to one another and appear to be perturbing each other as well as the state $1s\sigma 2s\sigma\,{}^1\Sigma g$ which is in the same neighbourhood. Their rotational structure resembles closely that of the "abnormal" ${}^1\Sigma g$ states $3\,{}^1K$, ${}^1L$, ${}^1M$ and ${}^1N$. Reasons are given which make it impossible to regard either of the two new states as states with only one electron excited. Two alternative methods of classifying the two new states and the seven other abnormal states which have transitions to $1s\sigma 2p\sigma\,{}^1\Sigma u$ and uniting them into a consistent scheme as states with both electrons excited are put forward and their merits discussed.

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